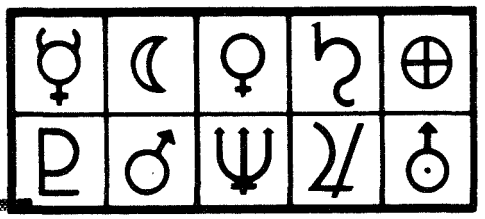

QR 21
JUNE 1971

PLANETARY QUARANTINE

SANDIA LABORATORIES QUARTERLY REPORT
PLANETARY QUARANTINE PROGRAM

Prepared by:
Planetary Quarantine Department 1740

SANDIA LABORATORIES

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SANDIA LABORATORIES QUARTERLY REPORT - PLANETARY QUARANTINE PROGRAM

TWENTY-FIRST QUARTERLY REPORT OF PROGRESS

For

Period Ending June 30, 1971

Planetary Quarantine Department
Sandia Laboratories, Albuquerque, New Mexico

Project No. 0064010

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SANDIA LABORATORIES QUARTERLY REPORT
PLANETARY QUARANTINE PROGRAM

Summary of Activities

Planetary Quarantine Analyses. In view of recent guidance from the Space Science Board (NAS) recommending less conservatism in planetary quarantine parameter value choices, and in view of the recent discovery of extremely heat resistant spores, studies have been undertaken which indicate that a revision of the philosophy guiding D-value choices is now appropriate. Accordingly, we have recommended that sterilization cycles be based on the estimated actual distribution of resistance among organisms on a spacecraft rather than upon the assumption that all organisms possess maximum known resistance. All available evidence indicates this approach is feasible. Additionally, the approach has the advantage of complete generality and:

(1) It requires the least possible sterilization time (for a fixed sterilant) while assuring that planetary quarantine requirements are met,

(2) the effects of environmental control, personnel control and cleaning can be assessed, and

(3) combinations of sterilants may be chosen in an optimal fashion to correspond to the variations in hardiness among organisms on a spacecraft.

This proposal for modifying the philosophy of D-value selection, as it was presented to PQAP, is outlined here.

Thermoradiation Experimentation. Activities this past quarter have centered on the investigation of the resistance of naturally occurring spores found in vacuum cleaner dust obtained from the AO Building Clean Room at Cape Kennedy. Knowing that very resistant spores exist in the soil near this building, it is obviously important to know whether these will be found in environmentally controlled spacecraft assembly areas also. From the results presented here, it appears that highly heat resistant spores will be found in such areas, but in a lower concentration than in completely open areas.

Thermoradiation Modeling. The previously developed model for thermoradiation inactivation of spores was used to analyze the inactivation of highly resistant soil spores. Tentative conclusions are:

(1) The thermal resistance of these organisms derives from their high thermal stability (very low entropy of activation) rather than from a change in chemical reaction energy.

(2) The nucleic acid molecular weight for highly radiation resistant soil spores is an order of magnitude or more less than that of B. subtilis var. niger.

(3) Free radical inactivation of highly resistant spores would appear to depend upon different species of free radicals than does the inactivation of B. subtilis var. niger.

Fine Particle Physics. Activities this past quarter were divided into two areas.

- The effects of relative humidity on particle adhesion to surfaces, and
- removal of organisms from dust particles.

The studies reported on here demonstrate that relative humidity has a striking effect upon dust particle adhesion to surfaces. As relative humidity increases, the forces of particle adhesion to surfaces increase dramatically - to a point, indeed, where standard spacecraft cleaning techniques become ineffective. This occurs even when the time period in which a particle on a surface is exposed to high relative humidity is short. As a general guideline, prior to sterilization, a spacecraft should not be exposed to relative humidities in excess of about 50%.

A study has been initiated to investigate means for separating naturally occurring organisms from dust particles. Currently, moderate success has been gained through floatation separation using Freon TF as the disassociation medium. It appears that Freon TF has no effect upon the viability of these spores.

Studies on Bacterial Spore Inactivation. In studying spore inactivation mechanisms, it was observed that formalin (aqueous formaldehyde) exhibited sporostatic properties very similar in character to those shown by alcohols in previous work. A description is given of a short study that was undertaken which demonstrated this sporostatic property of formalin, and a hypothesis as to the reasons is put forth.

Statistical Analysis of Experimental Data. As our experimental programs have increased, it has become desirable to have available a computer program for statistically analyzing reasonably large amounts of data.

During the past six months, such a program has been designed, coded and checked operationally. It is now in the process of being documented. The computer program is written in a very general language, and can be run on most computers. Decks or listings will be made available to interested persons.

Computerized Identification System. A completed version of the computerized bacterial identification system has been installed in the Lunar Information System at Cape Kennedy. Two subsidiary activities associated with the development of the identification system are reported on. The first is the discovery, using the system, that the third most frequently observed bacterial species found on Apollo spacecraft does not precisely correspond to any organism given in standard keys for the family to which it presumably belongs. The second is the development of a universally general bacterial identification code.

Federal Standard 209a. The formation of a Working Group to consider revision of Federal Standard 209a has begun. Preliminary contacts have been made with the Department of Defense, U. S. Navy, and U. S. Air Force for selecting Working Group members.

Planetary Quarantine Analyses

A. Description. This is a continuing activity undertaken on an as needed basis which is devoted to identifying planetary quarantine problems and determining ways in which such problems may be approached and resolved with minimal effect on planetary exploration.

B. Progress. Because of the recent evidence of concern over "hardy" soil spores, emphasis was placed during this past quarter in determining how much of a problem such spores might actually be in spacecraft sterilization. To do this it was first observed that a typical survivor curve for a randomly chosen population of naturally occurring organisms might be expected to look like that shown in Figure 1. Actual data of a similar naturally occurring spore population will be found in the next section of this quarterly report.

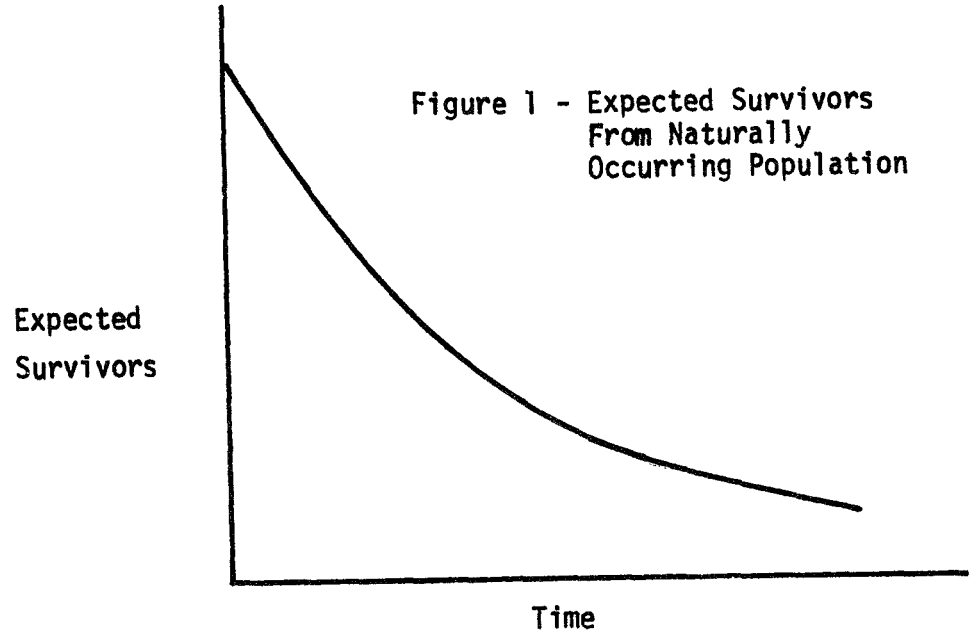


Figure 1 - Expected Survivors From Naturally Occurring Population

A curve such as that shown in Figure 1 is obtained for a population of naturally occurring organisms because the population is heterogeneous. This is illustrated in Figure 2, below, where it is imagined one is dealing with the total organism population on a spacecraft surface and its survival at 125°C. The total population, initially containing N_0 (about 10^5) organisms is imagined to be composed of

(1) N_1 expected vegetative cells (comprising about 90% of the total population) whose survival is shown as depending on a D-value, D_1, of about 3 minutes.

(2) N_2 expected spores (about 9% of the total population) whose D-value is D_2 (about 20 minutes),

(3) N_3 expected spores whose D-value, D_3, is approximately 1 hour, and

(4) N_4 expected spores whose D-value, D_4, is approximately 2 hours.

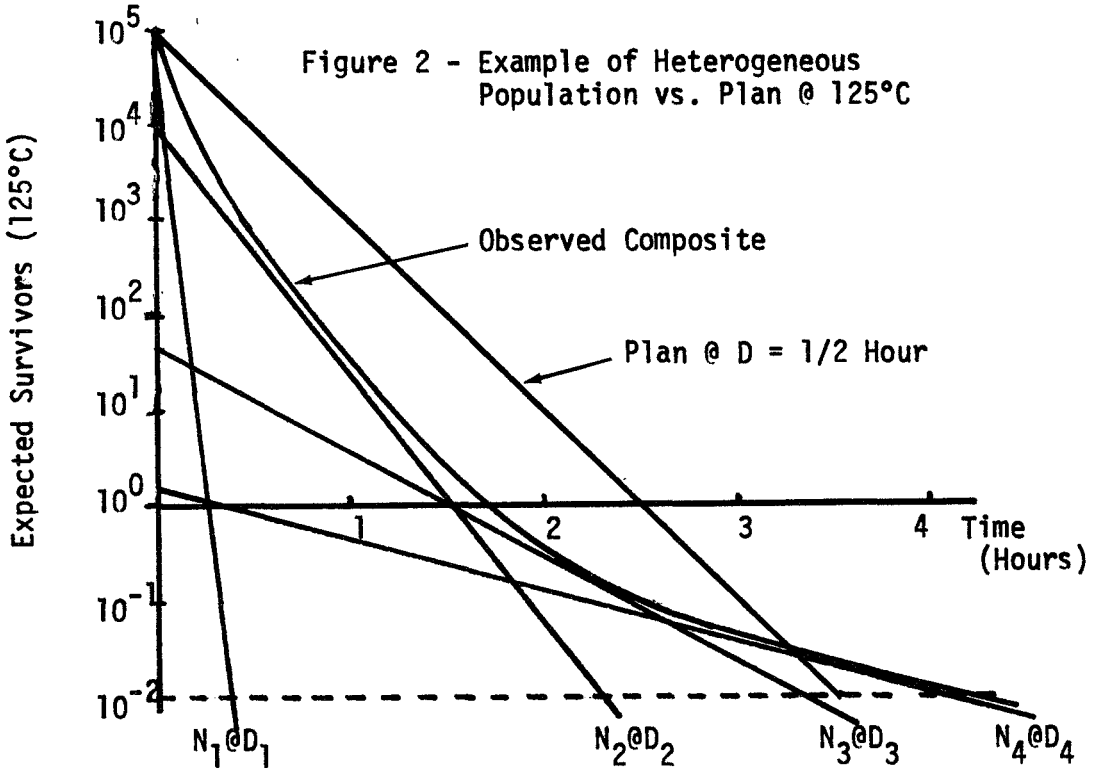


Figure 2 - Example of Heterogeneous Population vs. Plan @ 125°C

The survivor curve that would be seen experimentally for such a population is denoted "Observed Composite" in Figure 2, and is similar in character to that given in Figure 1. This hypothetical example given in Figure 2 is compared with the actual planned sterilization cycle for a population of 10^5 surface organisms on a spacecraft. The planned survivor curve is that shown with a 30 minute D-value for all 10^5 organisms (and thus a total sterilization time of 3-1/2 hours).

In this hypothetical example, the plan is inadequate because the actual expected number of survivors - in particular, the expected number of survivors from the population comprised of N_4 spores with a two hour D-value - exceeds 10^{-2} at 3-1/2 hours. On the other hand, a modest increase in sterilization time (to 4 hours) or a somewhat lower expected initial population N_4 would lead to an achievement of planetary quarantine objectives.

Thus, if one knew the actual distribution of hardiness of organisms on a spacecraft, that is, of N_0 organisms, N_1 have a D-value D_1, N_2 a D-value D_2, N_3 a D-value D_3, and so forth, it would be possible to assess the adequacy of current sterilization plans and determine the magnitude of changes needed if the plans proved to be inadequate.

To base new plans on an estimate of the distribution of the hardiness of organisms on a spacecraft, however, deviates from past planetary quarantine philosophy in which the established existence of organisms with a 2 hour D-value on a surface would imply the assumption of a 2 hour D-value for the total surface population - in other words, all organisms have, in the past, been assumed to possess the maximum known D-value. In the above example, this conservative approach would lead to a sterilization

time of 14 hours, or four times that currently planned. This also compares unfavorably with the theoretically adequate 4 hours just derived in Figure 2 for this example. The assumption of a maximum D-value for the total population is clearly an extremely conservative one.

In view of recent guidance from the Space Science Board urging less conservatism in parameter value choices, and in view of the recent discovery of extremely hardy spores, this seems an opportune time to consider revision of the philosophy guiding D-value choices. Rather than assume that a spacecraft population has a D-value compatible with the maximum D-value known at any given time, it seems appropriate to consider the possibility of estimating the variations in hardiness within the population and basing sterilization cycles on this estimate. To actually attain the required probability of spacecraft sterilization, one can do no better than this; in the sense that planetary quarantine requirements will be met in the least possible sterilization time.

Before adopting such an approach, one must, of course, question the practicality of attempting to estimate variations in hardiness in the microbial population on a spacecraft. Currently, this appears feasible. For example, recently published data indicate roughly that, when including vegetative cells, the frequency of occurrence of organisms isolated from an actual spacecraft decreases as the hardiness increases. This situation is illustrated in Figure 3. Here, between 85 and 90% of the organisms are assumed to be vegetative cells.

Shown also in Figure 3 is the probability P(D) that a random organism chosen from a given population will have a D-value of D (more precisely a probability of death given by $10^{-t/D}$). The graph of P(D) corresponds

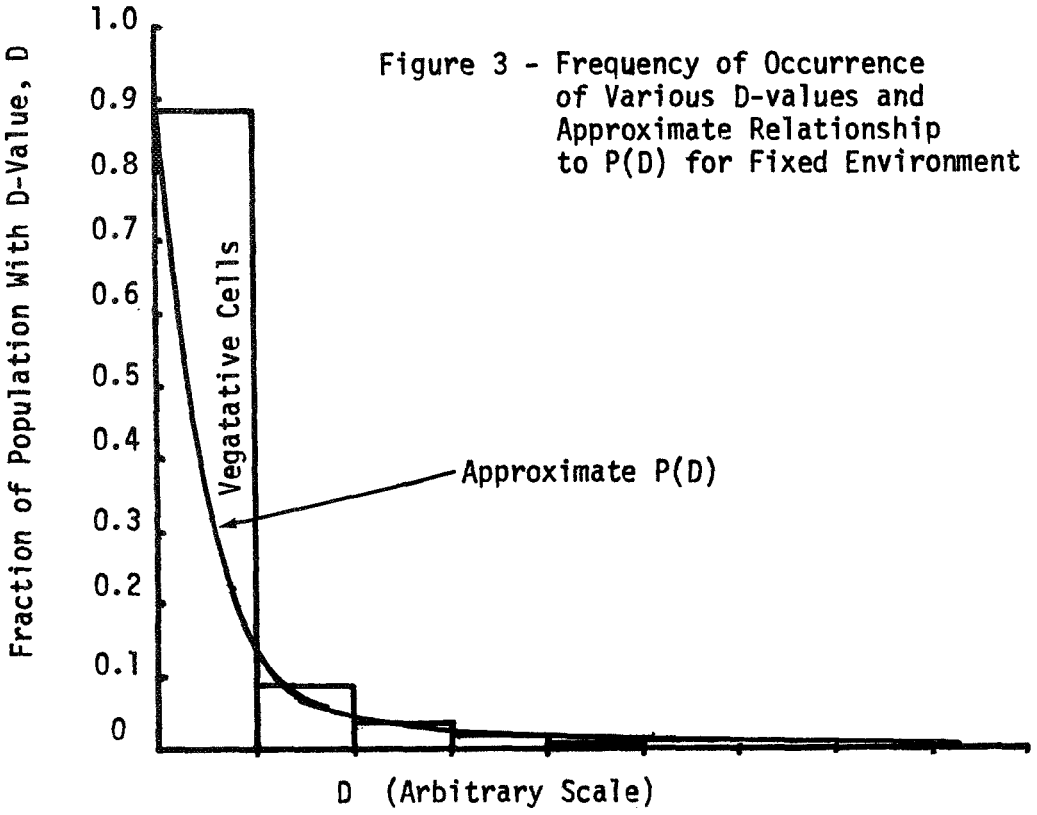


Figure 3 - Frequency of Occurrence of Various D-values and Approximate Relationship to P(D) for Fixed Environment

essentially to the fraction of times such organisms occur. Such a function, P(D), specifying the likelihood of encountering the D-value, D, is a measure of the variation in hardiness within a population. The question of practicality of estimating variations in hardiness can be translated into: Can P(D) is estimated?

Perhaps surprisingly, a fair amount of qualitative information and some quantitative information exists already - enough to feel hopeful about the feasibility of estimating P(D).

First, there exist data of the type already mentioned - frequency plots of varying hardiness of spacecraft isolates. The actual frequencies of different D-value ranges provide quantitative information. Qualitatively, these data indicate that, at least to a first approximation, P(D) is a monotonically decreasing function of D - that is, as D increases

P(D) goes down, so that one is less apt to encounter highly resistant organisms than to encounter easily killed ones.

Furthermore, there are data available that indicate that the function P(D) depends upon environment. Figure 4 illustrates this environmental dependency.

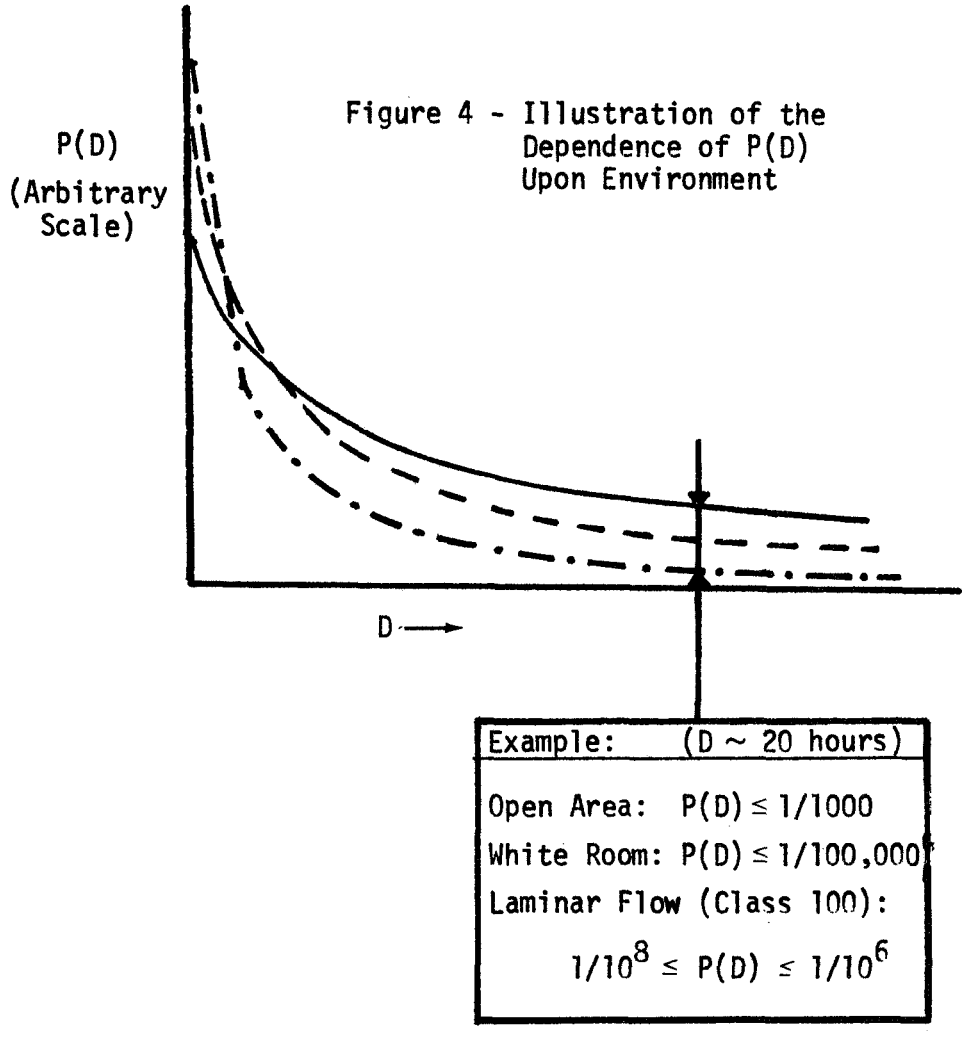


Figure 4 - Illustration of the Dependence of P(D) Upon Environment

In a "dirty" work area there is typically a higher proportion of spores to vegetative cells than in environmentally controlled areas. This proportion decreases as higher environmental control is exercised. In Figure 4, we have indicated one place (at D about 20 hours) where some reasonable quantitative information is available. Here we imagine perhaps 10 to 25% of the organisms in an open work area to be spores with 1/4 to 1% of those having a D-value of 20 hours based on reasonably conservative data currently available. Thus, in a dirty environment perhaps one in one-thousand organisms has a "D-value" of 20 hours. Based on Apollo sampling and our own spore heat studies of vacuum cleaner dust from assembly areas at the Cape (found next in this report) one anticipates perhaps 5% spores and perhaps only 1/5000 of these with a 20 hour D-value. Thus the estimate of one such hardy organism per 100,000 organisms in an area of moderate control (white rooms). In a highly controlled laminar flow area, soil spores (and therefore highly resistant ones) are even less likely - particularly if reasonable control procedures are used. Exact figures here are not known, but the range one hardy spore ($D \sim 20$ hours) per 10^6 to 10^8 seems very reasonable and perhaps conservative.

In further studies now under way, preliminary results indicate that such estimates of bacterial hardiness can be made reliably. Indeed there are several promising approaches to this problem of estimating variations in hardiness in microbial populations, and we feel that the feasibility of doing so can be established.

Finally, there is the question: If P(D) can be estimated, what effect might using the estimate to define sterilization cycles have upon

planetary exploration? In other words, what cycles might be anticipated? At this point in time a precise answer to this question is not known. In a rough qualitative way, it is our view that under certain conditions, current cycles could vary from marginal to somewhat inadequate. The 20 hour cross-section of Figure 4 is used to illustrate this in Figure 5.

In Figure 5, vegetative cells, spores with a nominal resistance of D = 20 minutes and very hardy soil spores at either 1 in 10^6 or 1 in 10^7 levels are assumed present. At a frequency of 1 in 10^6, the sterilization plan is inadequate. At 1 in 10^7, it is adequate. No account has been taken of spores with intermediate hardiness between 1/3 and 20 hours. In Figure 5, the marginal nature of current plans is visible. If spores with D-values of say 20 hours at 125°C can be expected 1 time in 10^6 organisms, then at least one log of these must be eliminated (based on an expected number of 10^{-1} in 10^5 organisms), and this requires 20 hours at 125°C. Should something like this actually occur, one might wish to consider the use of thermoradiation to attain this required single log decrease in the very hardy spores. Total adequate sterilization could then be accomplished, in this example, by actually using <u>less</u> than the <u>planned</u> sterilization time of 3.5 hours with the addition of at most 35 krads total dose of γ-radiation.

In the more general case (including the <u>complete</u> distribution of hardiness) we would anticipate that this same approach is entirely feasible.

The four major advantages that appear to accrue from using estimates of the actual distribution of hardiness of organisms on a spacecraft are:

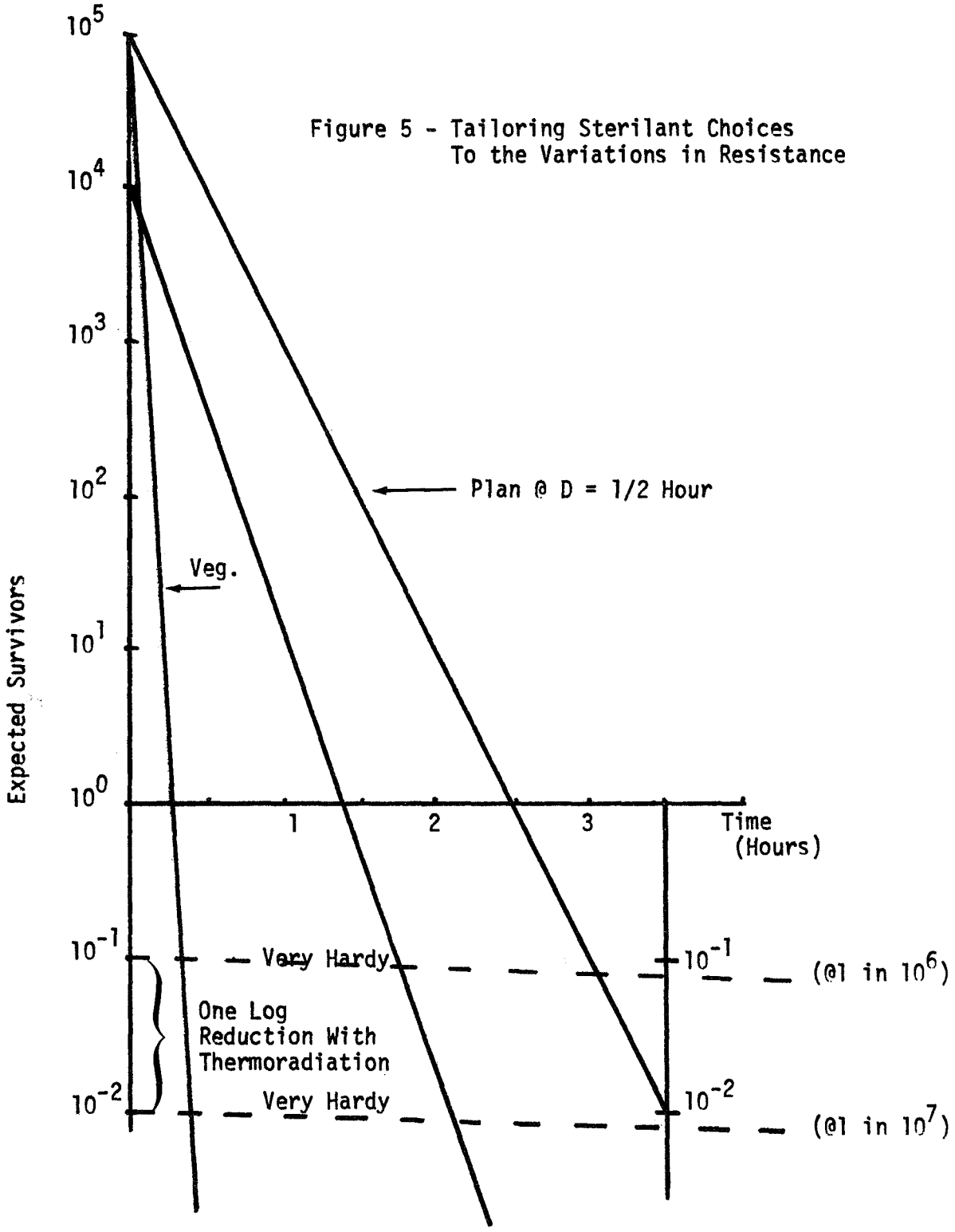


Figure 5 - Tailoring Sterilant Choices
To the Variations in Resistance
Expected Survivors
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
10^{-2}
Plan @ D = 1/2 Hour
Veg.
1
2
3
Time
(Hours)
Very Hardy
One Log
Reduction With
Thermoradiation
Very Hardy
10^{-1}
(@1 in 10^6)
10^{-2}
(@1 in 10^7)

(1) Acknowledging that variations in hardiness exist, this approach requires the least possible sterilization time (for fixed sterilant) while guaranteeing that planetary quarantine requirements are met.

(2) The effects of environmental control, personnel control, and cleaning can be assessed, and as more control and cleaning are used, the less likely is one to encounter problems from very hardy organisms.

(3) Sterilization means can be tailored to the distribution - as illustrated by the introduction of a modest thermoradiation treatment in the above example to effectively sterilize only a small portion of the total population.

(4) This approach is completely general. Although the parameter values (that is those defining P(D)) may change as circumstances change, the approach, itself, need never be altered. Thus, with the adoption of this approach, all new information related to bacterial resistance may be incorporated without requiring any change in philosophy guiding the planning of spacecraft sterilization cycles.

During this past quarter, this approach for determining sterilization cycles was recommended by us to the Planetary Quarantine Advisory Panel and the Planetary Quarantine Officer, NASA. A document giving details of this recommended approach is being prepared.

Thermoradiation Experimentation

A. Description. The objective of this activity is to thoroughly investigate the sterilizing effects of combinations of heat and ionizing radiation and to assess the practicality of this process for spacecraft sterilization. Thermoradiation may offer the possibility of sterilization at temperatures less than 100°C at low dose rates of approximately 10 krad/hour. This is possible because of a synergistic effect in bacterial inactivation which has been observed when combinations of heat and gamma radiation are applied simultaneously. Should any spacecraft components prove to be heat sensitive at high temperatures, thermoradiation offers a potential means of overcoming reliability problems. In addition, it provides a very effective means of inactivating highly resistant naturally occurring spores.

B. Progress. In an effort to further understand the effect of inactivation mechanisms on naturally occurring spores, several experiments were conducted using dry heat and thermoradiation as the inactivating agents. The primary difference between these and former experiments with naturally occurring organisms, described in QR-20, was the source and state of the dust. The dust for these tests was collected with a vacuum cleaner in the AO Building Clean Room at Cape Kennedy by the Spacecraft Bioassay Unit, PHS, and was maintained in a dry condition through exposure to dry heat and thermoradiation.

Matherials and Methods. After the debris was removed, the dust was sieved to a size of less than 147 microns. Each sample was prepared by weighing 0.1 $\pm$ .001 gram of soil on an analytical balance and placing the soil in a dish-shaped disc of aluminum foil 1.25-inch in diameter and 0.060-inch deep in the indented portion. A flat foil

disc was used as a cover. Four samples were then placed between two aluminum strips, 1.5-inches wide by 0.020 thick. The completed assembly was held together with wire clamps. The assembled sample strips were then placed in a desiccator over Drierite for 15 hours prior to exposure to the inactivating environments.

For dry heat exposure, the sample strips were placed in a recirculating air oven at 125°C and an ambient RH of approximately 30% at 22°C. The same arrangement was used for thermoradiation exposure, except that the oven was placed inside the Gamma Irradiation Facility (GIF) cell an appropriate distance from the cobalt-60 source. The thermoradiation environment consisted of a temperature of 125°C and a gamma radiation dose rate of 77 krads/hr. Silver phosphate dosimeters were placed on selected sample strips to verify the computed dose rate.

After exposure, the strips were dissembled and each soil sample, including the two foil discs, was placed in a separate 50 ml beaker containing 10 ml of sterile, 0.1 percent Tween 80 water. The samples were then insonated for 2 minutes to remove any organisms from the foil discs and to disassociate the organisms from the dust particles. Each sample strip containing four replicate samples represents a single data point. Additional ten-fold serial dilutions were made as required. Dilutions were plated out in duplicate in Trypticase Soy Agar with 0.1% soluble starch and 0.2% yeast extract added as described in the 26th Quarterly Report of the Public Health Service Laboratory, Phoenix. The plates were both underlayed and overlayed with the same type of media. The plates were incubated at 32°C and counted after 6 days incubation.

Results. D-values were derived from a least squares fit of the resistant subpopulations (see Statistical Analysis of Experimental Data, a later section in this report). The dry heat curve, shown in Figure 6, indicates a D_{125} value of 8 hours. While this varies significantly from the 29.45 hour D_{125} value for the PHS, Phoenix stock, the thermoradiation D-value of 57 minutes is comparable with that of 1.04 hours for the PHS, Phoenix stock that was reported last quarter (QR-20). The thermoradiation data is shown in Figure 7.

In any event, these organisms have much higher D-values than Bacillus subtilis var. niger. The significant conclusion to be drawn from these experiments is that highly heat and thermoradiation resistant organisms were found in the dust from the conventional clean room spacecraft assembly areas of Hangar AO, although their frequency is lower in this area where some environmental control is exercised.

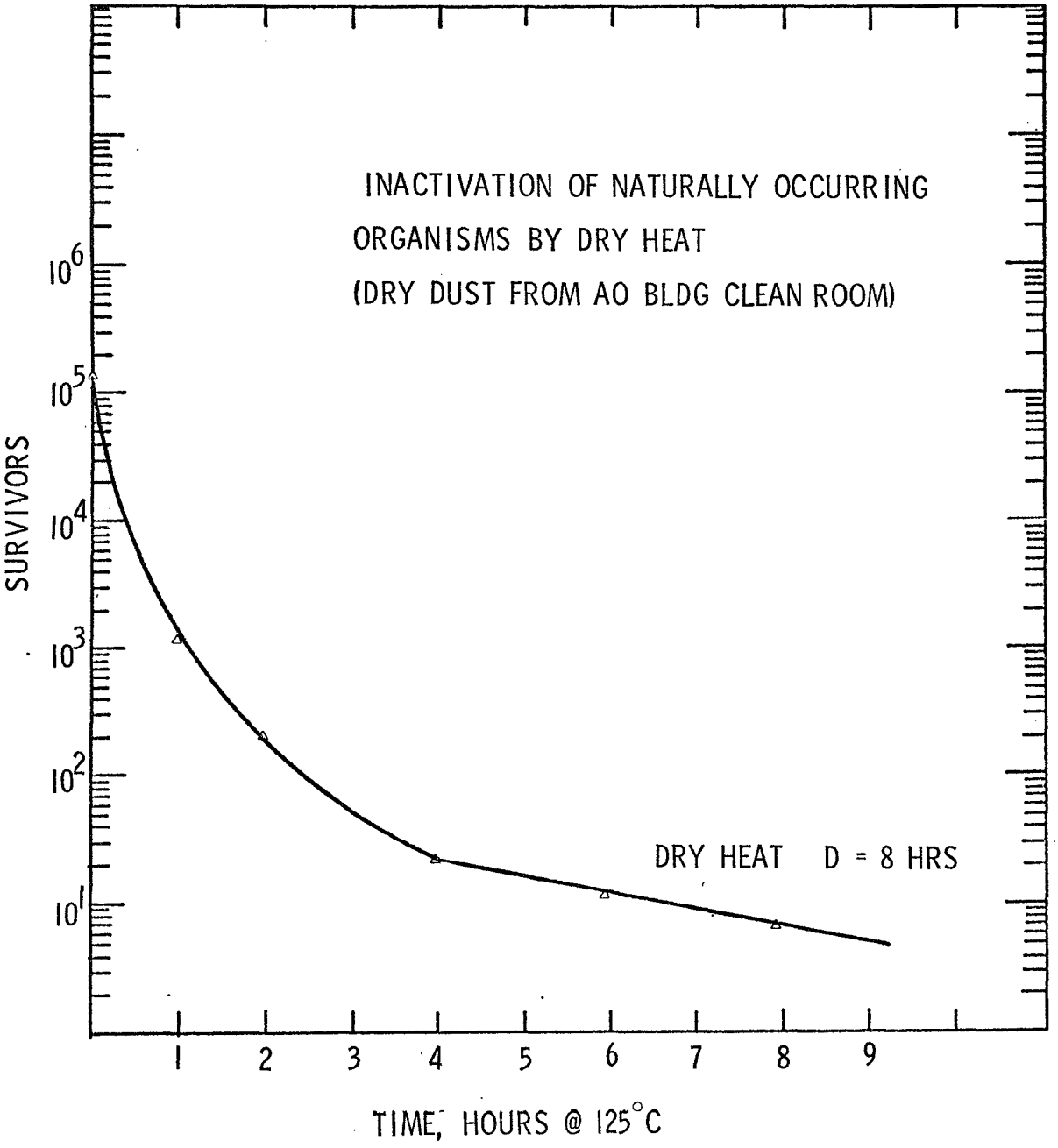

INACTIVATION OF NATURALLY OCCURRING
ORGANISMS BY DRY HEAT
(DRY DUST FROM AO BLDG CLEAN ROOM)
SURVIVORS
10^6
10^5
10^4
10^3
10^2
10^1
DRY HEAT D = 8 HRS
1 2 3 4 5 6 7 8 9
TIME, HOURS @ 125°C

Figure 6

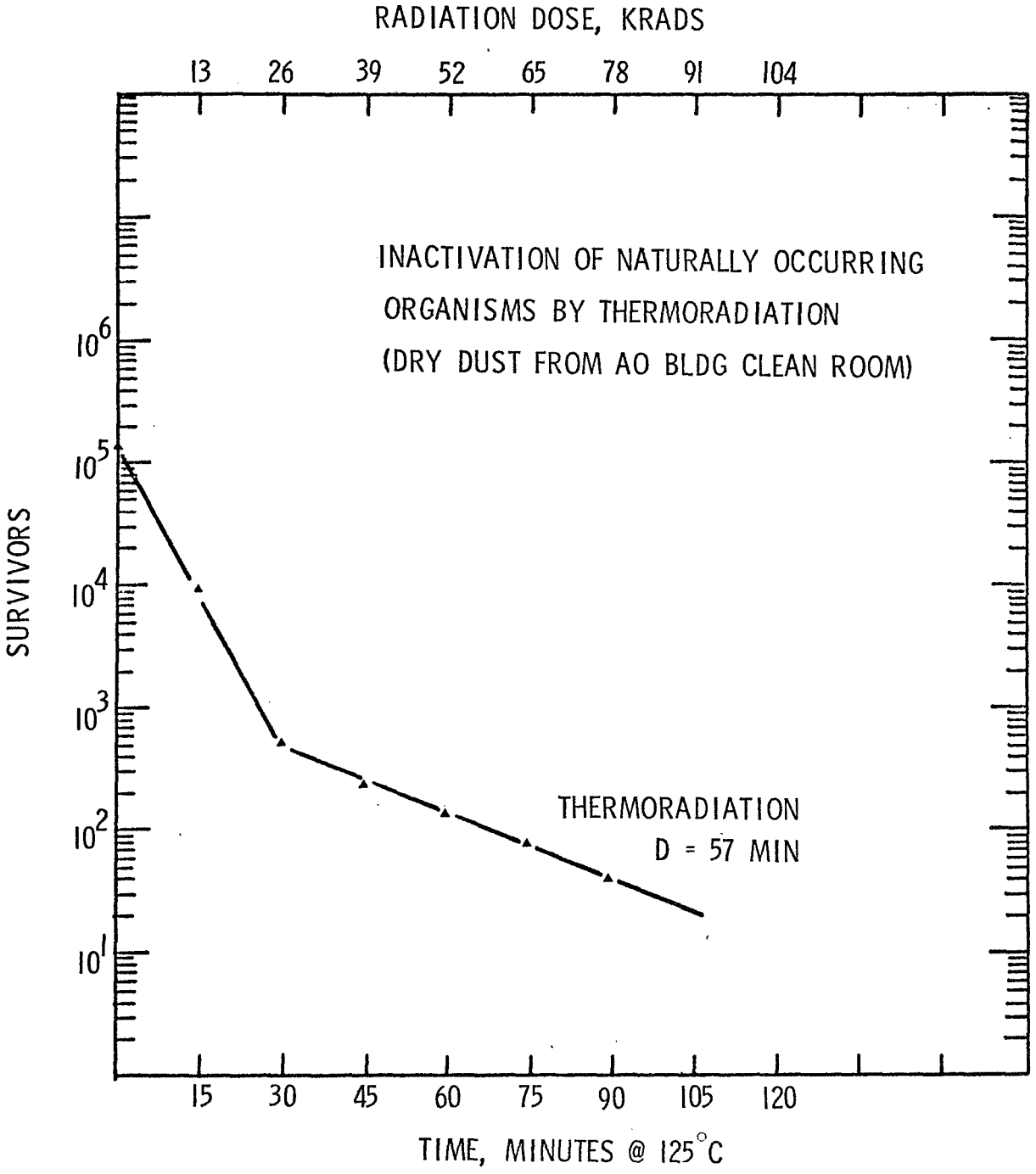

RADIATION DOSE, KRADS
13
26
39
52
65
78
91
104
INACTIVATION OF NATURALLY OCCURRING
ORGANISMS BY THERMORADIATION
(DRY DUST FROM AO BLDG CLEAN ROOM)
SURVIVORS
10^6
10^5
10^4
10^3
10^2
10^1
THERMORADIATION
D = 57 MIN
15
30
45
60
75
90
105
120
TIME, MINUTES @ 125°C

Figure 7

Thermoradiation Modeling

A. Description. The basic model utilized is that described in previous quarterly reports, QR-19 and QR-20.

Briefly, this model expresses expected survivors at time t, E(N(t)), as

$$E(N(t)) = N(0)\, e^{-kt} \quad ,$$

where k has the form

$$k = k_T + k_R + k_{TR} \quad .$$

The parameter k may be considered a reaction rate constant with k_T, k_R and k_{TR} rate constants for inactivating chemical reactions promulgated by temperature, radiation and thermoradiation respectively. These rates are given by

$$k_R = Cr_d \qquad \text{with } C = 2.398 \times 10^{-6}\ (\omega)^{0.481} \tag{1}$$

where ω is the organism nucleic acid molecular weight (Daltons) and r_d is radiation dose rate (see QR-20);

$$k_T = \frac{KT}{h}\ \exp\ (-\Delta F^{\neq}/RT), \tag{2}$$

with

$$\Delta F^{\neq} = \Delta H^{\neq} - T \cdot \Delta S^{\neq}$$

obtained from absolute reaction rate theory as described in previous reports; and

$$k_{TR} = e^{\alpha} r_d^{\beta/T} e^{-\gamma/RT} \quad . \tag{3}$$

B. Progress. The parameters α, β, γ, $\Delta H^{\neq}$, $\Delta S^{\neq}$ and C have been determined for the naturally occurring spores, PHS, Phoenix stock. Table 1 shows the parameters for this stock compared with B. subtilis parameter values.

Parameter	B. subtilis	PHS Spores
C	0.02336	0.011234
α	16.27	28.6
β	159.1	360.9
γ	12944	25179
$\Delta H^{\neq}$	33.5 k cal/mole	23.5 k cal/mole
$\Delta S^{\neq}$	12.63 e u	-20.6 e u

With these base line parameters it is possible to determine expected D-values for the PHS stock as a function of temperature and dose rate. This is indicated in Figure 8.

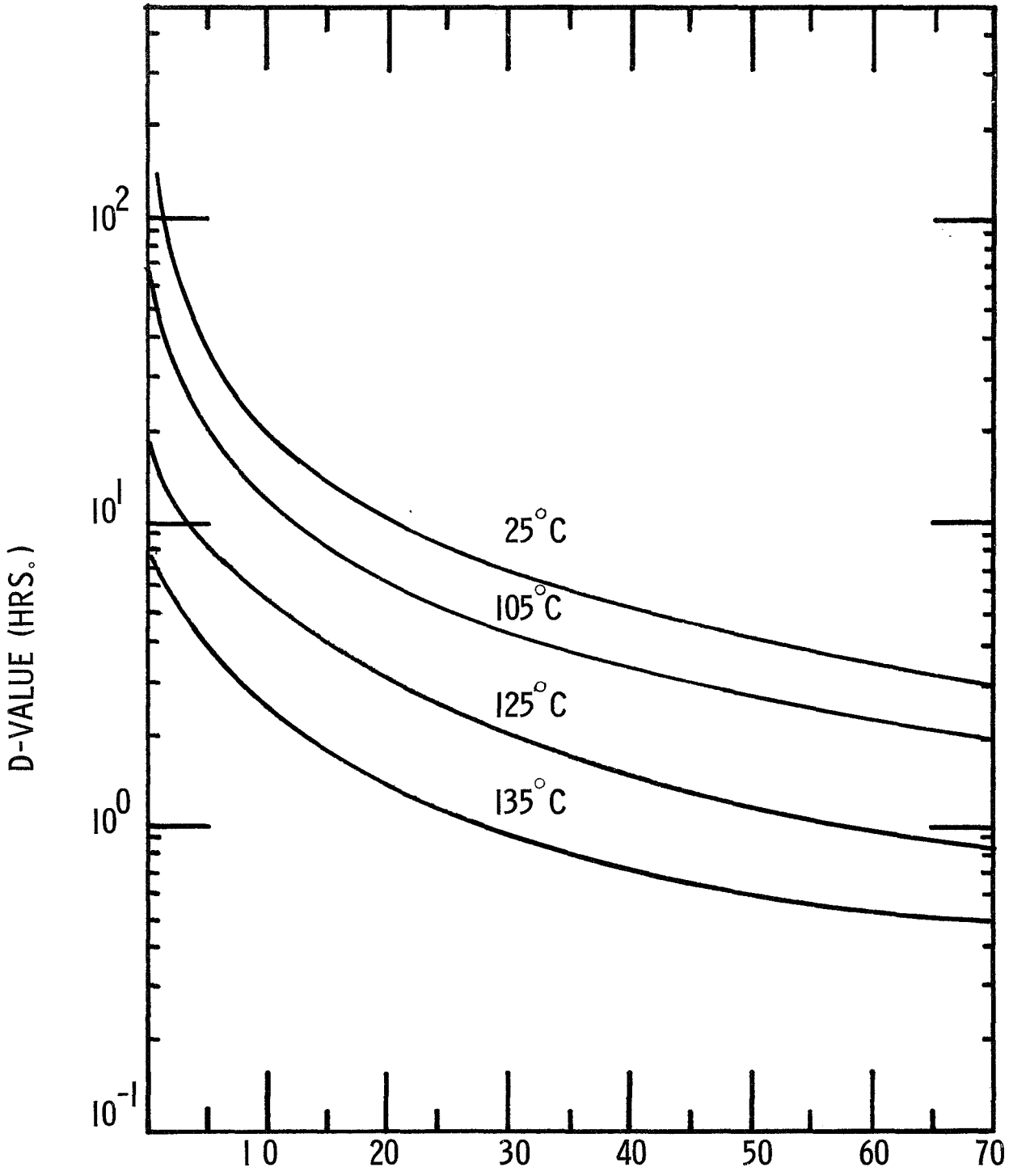
Some tentative observations which can be made concerning the PHS Phoenix stock include:

a. The very low entropy of activation value, $\Delta S^{\neq}$, for the PHS spores indicates an extremely heat stable molecule; the implication being that the high thermal resistance of

of these spores derives from some mechanism conferring stability to vital spore constituents (as opposed to some gross change in reaction taking place).

b. On the other hand, from the values of α and γ it begins to appear that for free radical mediated processes the entropies of activation for the B. subtilis and PHS spores are about the same, and the greater resistance of the PHS spores to thermo-radiation (k_{TR} term) is attributable to a change in reaction.

c. From (1), the apparent molecular weight of the PHS spore nucleic acid, using radiation alone, is 4.3×10^7 Daltons. For B. subtilis the comparable value is 1.96×10^8 Daltons. It is interesting to observe that, from these preliminary data, the apparen molecular weight of the nucleic acid for the PHS stock is approximately the same as the value for T1 phage.


D-VALUE (HRS.)
10^2
10^1
10^0
10^{-1}
25°C
105°C
125°C
135°C
10
20
30
40
50
60
70
DOSE RATE (KRADS/HR)
Figure 8

Fine Particle Physics

A. Description. This is a study of small particle behavior related to microbial burden of spacecraft surfaces prior to final sterilization. Particles that have highly resistant microorganisms attached to them are of prime interest to this study. Since such particles are very few in number compared to total particle contamination, they are very difficult to identify for study. For this reason, total particle contamination is being studied relative to particle accumulation and retention on surfaces. Only a small number of particles bearing very hardy microroganisms would be expected to accumulate on a spacecraft during assembly in a very clean area; however, one to ten percent of the type of microorganism reported by Favero in USPHS Report No. 32 dated January 1971, could survive a 24-hour heat cycle at 125°C. In order to identify factors whose control would lead to a lower particulate burden (and hence lower bioburden) we believe that a rigorous analysis of spacecraft surface particle loading is necessary.

B. Progress.

The Effects of Relative Humidity on Particle Adhesion to Surfaces.

During this quarter a series of experiments was conducted to determine the effects of relative humidity on particle contamination adhesion to surfaces. Almost all naturally occurring particles are affected in some manner by water vapor in the surrounding air, particularly as the water content of air increases. Of special interest are those particles that pick up moisture from the air which partially or totally dissolve and

then form a very strong bond with the surface on which the particles are located. At elevated humidities this occurs quickly - in a very few minutes. These particles cannot be effectively removed by dry wipes, vacuum cleaning or other cleaning methods that are permitted on many spacecraft surfaces. Thus, the final microbial burden of a spacecraft can be affected by the relative humidity of the air in which it was assembled.

These experiments were set up to study relative humidity effects from 33 percent to one hundred percent. Glass dessicators containing the following saturated salt solutions were used for conditioning chambers.

H_2O	Water	100%
$NH_4H_2PO_4$	Ammonium Phosphate	93%
KBr	Potassium Bromide	84%
NaCl	Sodium Chloride	76%
$NaNO_2$	Sodium Nitrite	66%
$Na_2CR_2O_7 \cdot 2H_2O$	Sodium Dichromate	52%
$MgCl_2 \cdot 6H_2O$	Magnesium Chloride	33%

Test particles were used that simulated as nearly as possible the type particles expected in a clean room environment. Test particles were obtained by sieving building vaccum cleaner dust to exclude particles larger than 140 microns. After sieving, the test particles were stored in dry air over a dessicant bed until use.

Test surfaces were 1" x 1" highly polished metal foils cemented to 1 x 3 inch glass microscope slides. Test surfaces were etched to

permit photographing the exact same area before and after "blowoff" (a simulated environmental removal of particles to test retention ability).

A 3.3 cu. ft. particle loading chamber was used to load the test surfaces prior to conditioning at the various relative humidity levels. An agitator fan was located near the bottom of the chamber and a glass tube was used to feed test particles into the fan inlet during loading. A horizontal rack was positioned in the upper half of the loading chamber to hold test slides during loading.

A blowoff fixture was used to retain test slides during "blowoff." The fixture consisted of a 1/8" diameter jet located 1/2" above the test strip. Dry nitrogen was used as the blowoff gas which was controlled by a solenoid valve and timer. Nitrogen pressure was regulated to 20 psi during blowoff.

The microscope-camera system is a Leitz Ortholux equipped with Leitz Ultra-Pak vertical illumination equipment. This combination with the highly polished foils provides an excellent high contrast dark field illumination system. The system will resolve particles less one micron size. A 4" x 5" Polaroid camera back is used for photographing test slides. A magnification of 90x is used for photographs to be counted and higher magnifications for individual particle analysis.

Experimental Procedure. The following sequence of steps was followed during the experiment.

(1) 12 clean slides were placed on the loading chamber rack.

(2) The timer was set for 60 seconds to start blower.

(3) 2 ml (approximately 1.2 gm) test particles were released into the loading tube during first 30 seconds of the loading cycle.

(4) After the load cycle, the 12 test slides were carefully removed and placed in the humidity control chamber. Every effort was made to avoid air currents, drafts, vibration and rapid movement of the test slides during handling. One slide was removed from the humidity control chamber after each of the following conditioning periods: 84%, 93%, and 100% - 5, 10, 15, 30 minutes; 1, 2, 4, 8, 24, 48, 72 hours. 33%, 52%, 66%, 76% - 30 minutes; 1, 2, 4, 8, 24, 72, 200, 720 hours.

(5) After removal from the humidity control chamber, each slide was immediately photographed. Then it was exposed to a "blowoff" treatment for 10 seconds at 20 psi. (Following "blowoff" it was immediately rephotographed. Two separate defined areas were photographed before and after "blowoff").

(6) Particles, as recorded on the photographs, were sized and counted in ranges of (a) less than 10 microns, (b) 10 microns and larger, and (c) 50 microns and larger. The lower limit of particle size count was approximately 1 micron. The area photographed from each slide was approximately 1.6 mm^2. Approximately 120 particles were counted per 1.6 mm^2 before blowoff (initial load).

(7) Four test slides were loaded, photographed, then subjected to "blowoff" procedure, rephotographed, and counted for reference or control.

Results. Particle count data was converted to "Percent Particles Remaining After Blowoff," designated as "Retention %" and defined as

$$\text{Retention \%} = \frac{\text{Count after blowoff}}{\text{Count before blowoff}} \times 100$$

Data are presented in graph form in Figures 9 through 15 for individual relative humidity levels. Results are plotted as "Retention %" versus the time (in hours) of conditioning at the various humidity levels. At relative humidity levels of 76%, 84%, 93% and 100%, "Retention %" is plotted for the particle size categories "total particles", "10 microns and larger," and "50 microns and larger," marked as curves A, B and C. The "50 microns and larger" is not shown for relative humidity levels below 76% since there was very little retention of large particles at these humidities. Figure 16 shows a comparison plot of total particles for all relative humidity levels.

This series of experiments indicates that the major effect of relative humidity on particle retention occurs within one hour at any of the relative humidity levels investigated. At higher humidity levels, particles become firmly attached to the surface in a few minutes. Disassociation of the particles occurred at all humidity levels; (occurring much more rapidly at the higher humidity levels). The disassociation or "breakup" of particles left large numbers of small particles adhering to the test surface that were much harder to remove than the original, or parent, particles.

These experiments show relative humidity to be a very important factor in surface particle retention which relates directly to total

loading factors for a spacecraft. The hygroscopic property of particles- the ability to pick up moisture from air - appears to be a major factor in the adhesion of particles to surfaces. Molecular, electrostatic and other forces account for only approximately 20% of the test particles retained on the test strips.

As a result of this study, it may be seen that particle removal (and therefore bioburden loss) from a spacecraft surface is most easily facilitated either by cleaning or natural environmental removal factors when the surface has not been exposed to high humidity environments. In particular, it would appear that spacecraft surfaces should not be exposed to environments with relative humidity above about 50% for even short periods of time.

Removal of Organisms from Dust Particles. A study has been initiated to investigate the means for separating naturally occurring organisms from dust particles. This was prompted by the need to ascertain the microbial loading of spacecraft from airborne dust particles indigenous to the areas in which actual assembly and test operations are performed. Of particular interest are the organisms which are highly resistant to heat.

For the purpose of this study, about one liter of dirt was obtained by the Spacecraft Bioassay Unit, PHS (Phoenix) from several locations outside and adjacent to the AO Building at Cape Kennedy. After the larger pieces of debris were removed, the dirt was sieved to a size of less than 147 microns. This size was selected to permit study of some of the relatively larger particles as well as the very fine dust.

The entire amount of dust was then tumbled for 11 hours to mix it thoroughly and to provide a homogeneous sample stock.

The liquid selected as the disassociation medium was Freon TF. Among the reasons for this selection were:

a. The density of Freon TF is 1.55, whereas the density of spores is approximately 1.2 - 1.3. It was believed that this difference in physical properties would be beneficial in floating spores to the top of any Freon/dust solution.

b. The viscosity of Freon TF is 0.682 centipoises at 25°C. The viscosity of other candidate liquids at the same temperature is 0.894 for water and about 1.265 for 95% ethanol. This factor should facilitate both separation and filtering.

c. The solvent properties of Freon TF would dissolve any oily films present which would act as a binder between the particles and organisms.

d. Freon TF is readily available commercially.

Since information was not generally available on the effect of Freon TF on organisms, a series of experiments was conducted to determine what effects, if any, it would have on growth and survival characteristics. One gram dust samples were weighed and deposited in bottles containing 50 ml of Freon TF. At the end of periods 0, 5, 7, 12 and 14 days, the sample solution was insonated and appropriate serial dilutions were prepared and plated in Trypticase Soy Agar with 0.1% soluble

starch and 0.2% yeast extract added. The plates were underlayed and overlayed with the same media and incubated for five days at 32°C. There was no discernable difference in plate counts among the samples or between these samples and similar sample solutions containing only sterile deionized water. Since our proposed experimentation would demand that the dust be in the Freon TF for only short periods of time, it was concluded that the Freon would have no detrimental effects on the spores.

Only a few experiments were conducted this quarter. While heat resistant organisms were separated from dust particles, further experimentation is needed to increase the yield of these organisms and to refine the laboratory procedures.

EFFECTS OF RELATIVE HUMIDITY ON SURFACE PARTICLE RETENTION

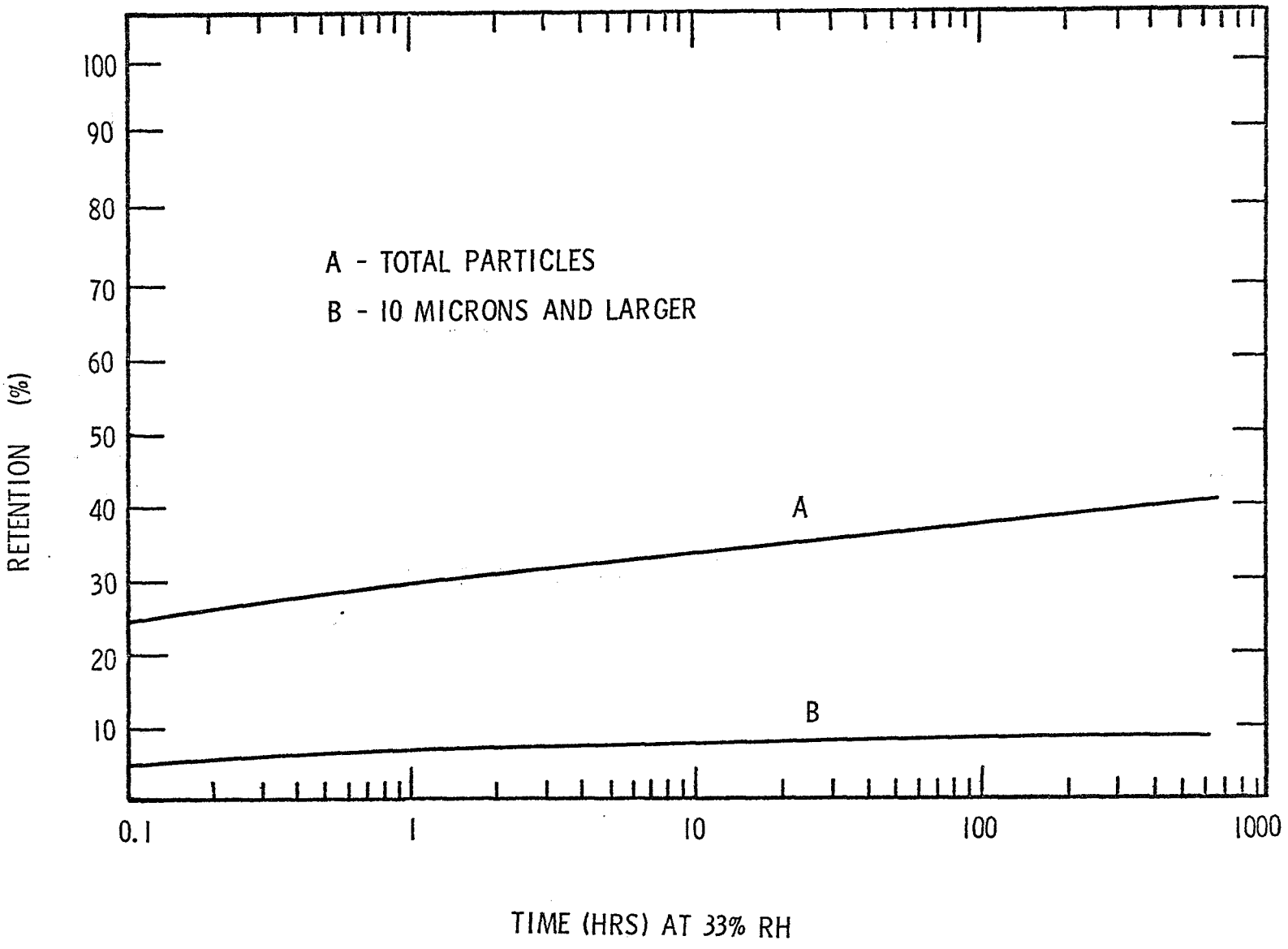


Figure 9

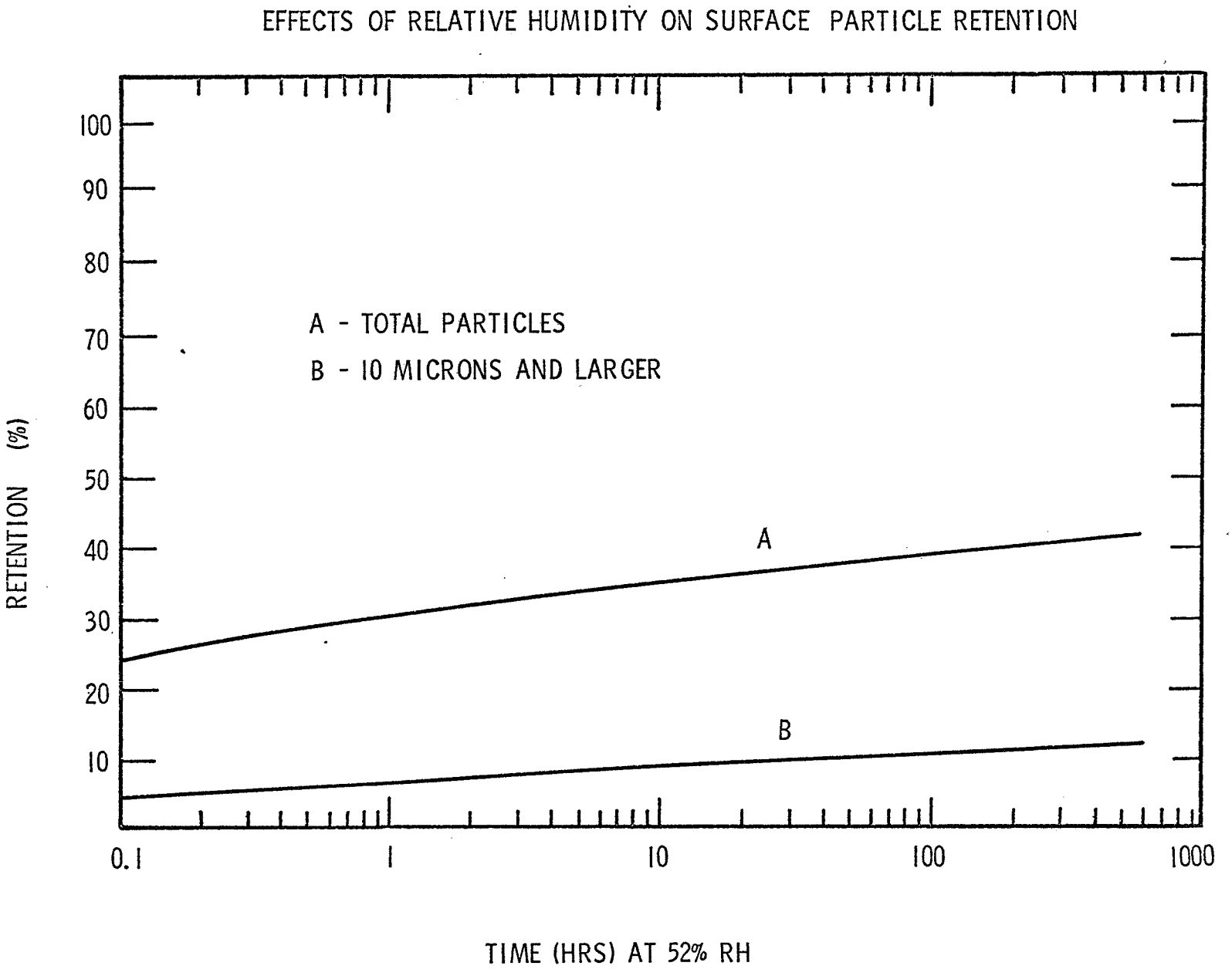

EFFECTS OF RELATIVE HUMIDITY ON SURFACE PARTICLE RETENTION
A - TOTAL PARTICLES
B - 10 MICRONS AND LARGER
A
B
RETENTION (%)
100
90
80
70
60
50
40
30
20
10
0.1
1
10
100
1000
TIME (HRS) AT 52% RH

Figure 10

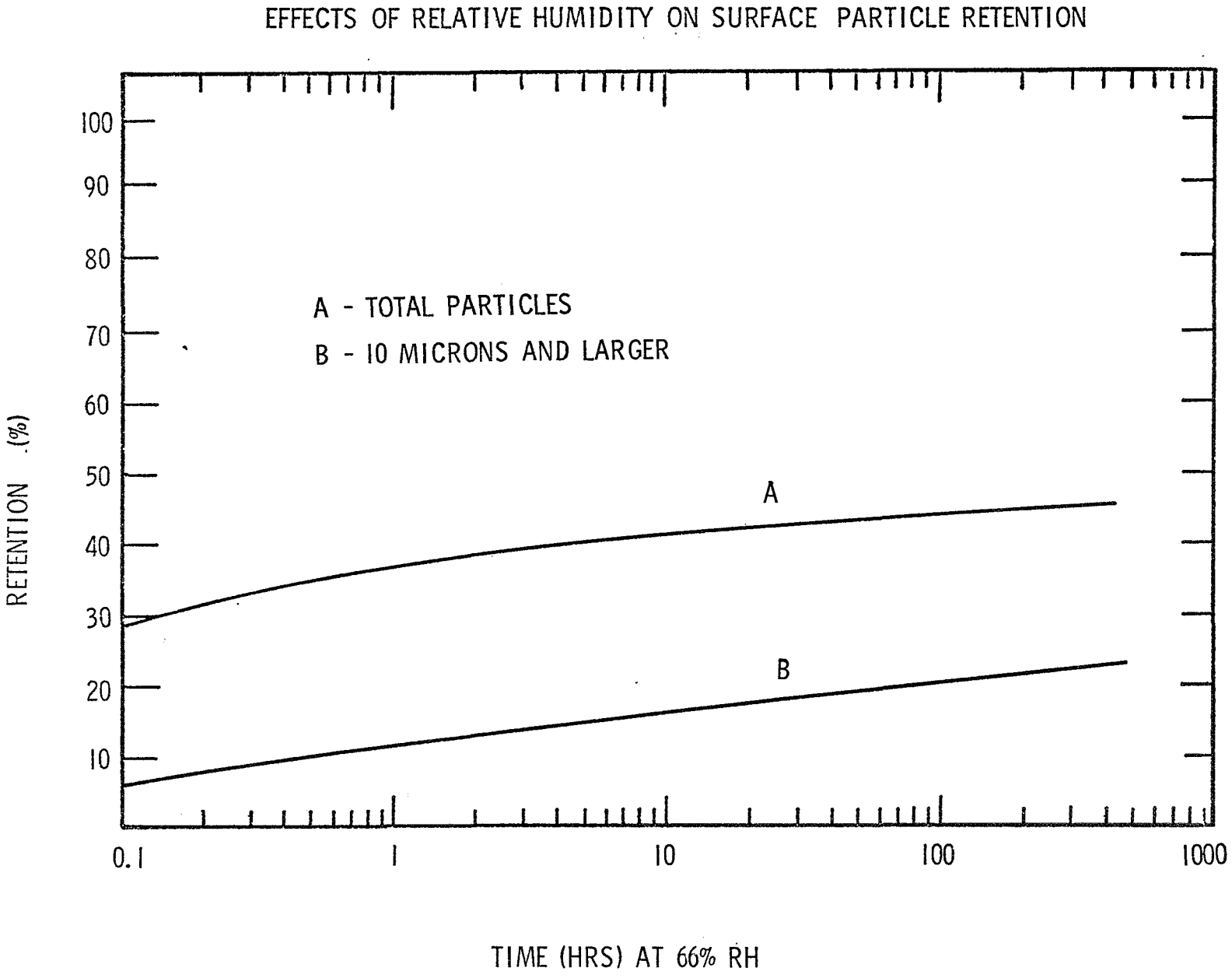

EFFECTS OF RELATIVE HUMIDITY ON SURFACE PARTICLE RETENTION
A - TOTAL PARTICLES
B - 10 MICRONS AND LARGER
A
B
RETENTION (%)
100
90
80
70
60
50
40
30
20
10
0.1
1
10
100
1000
TIME (HRS) AT 66% RH

Figure 11

EFFECTS OF RELATIVE HUMIDITY ON SURFACE PARTICLE RETENTION

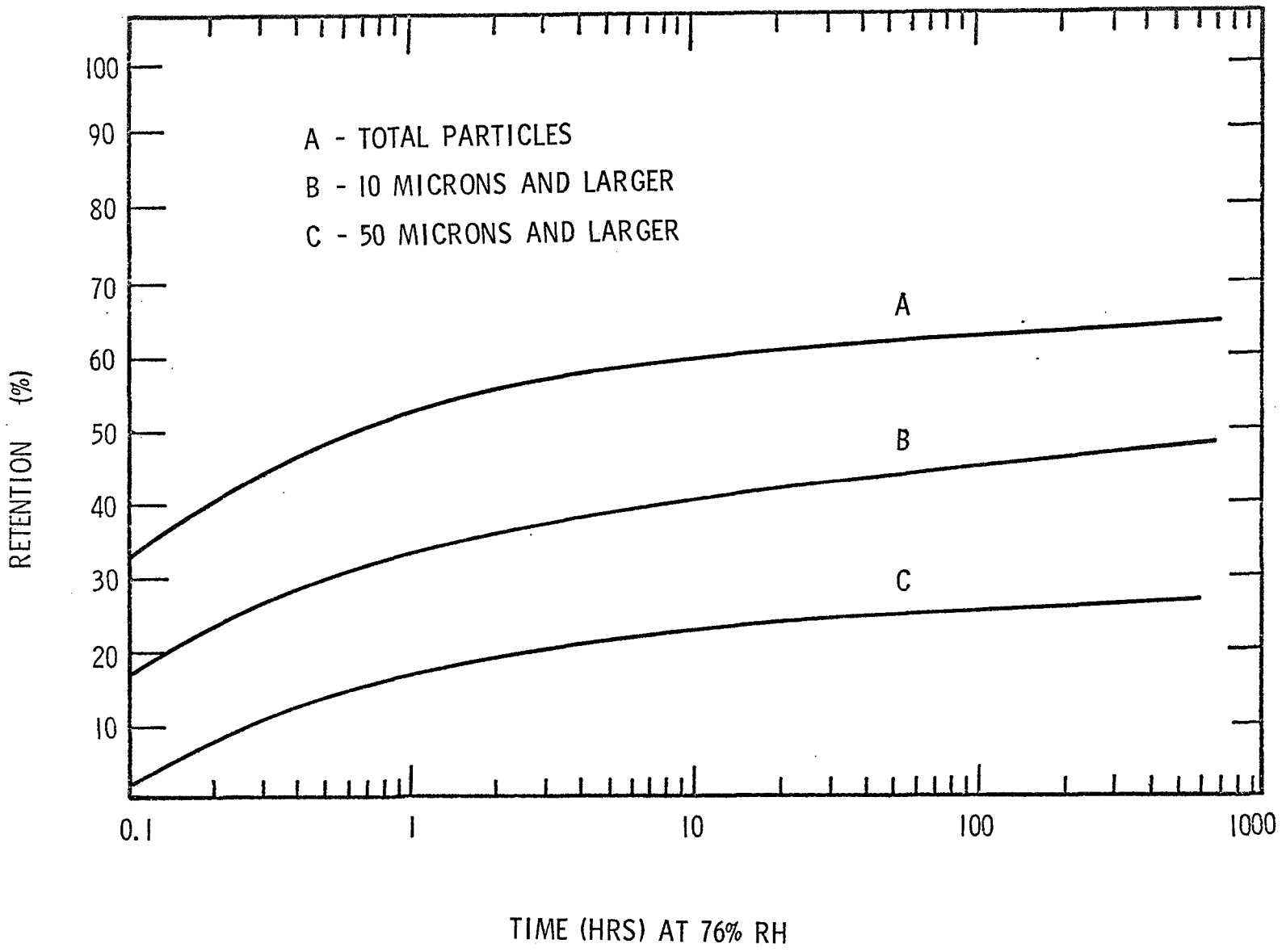


Figure 12

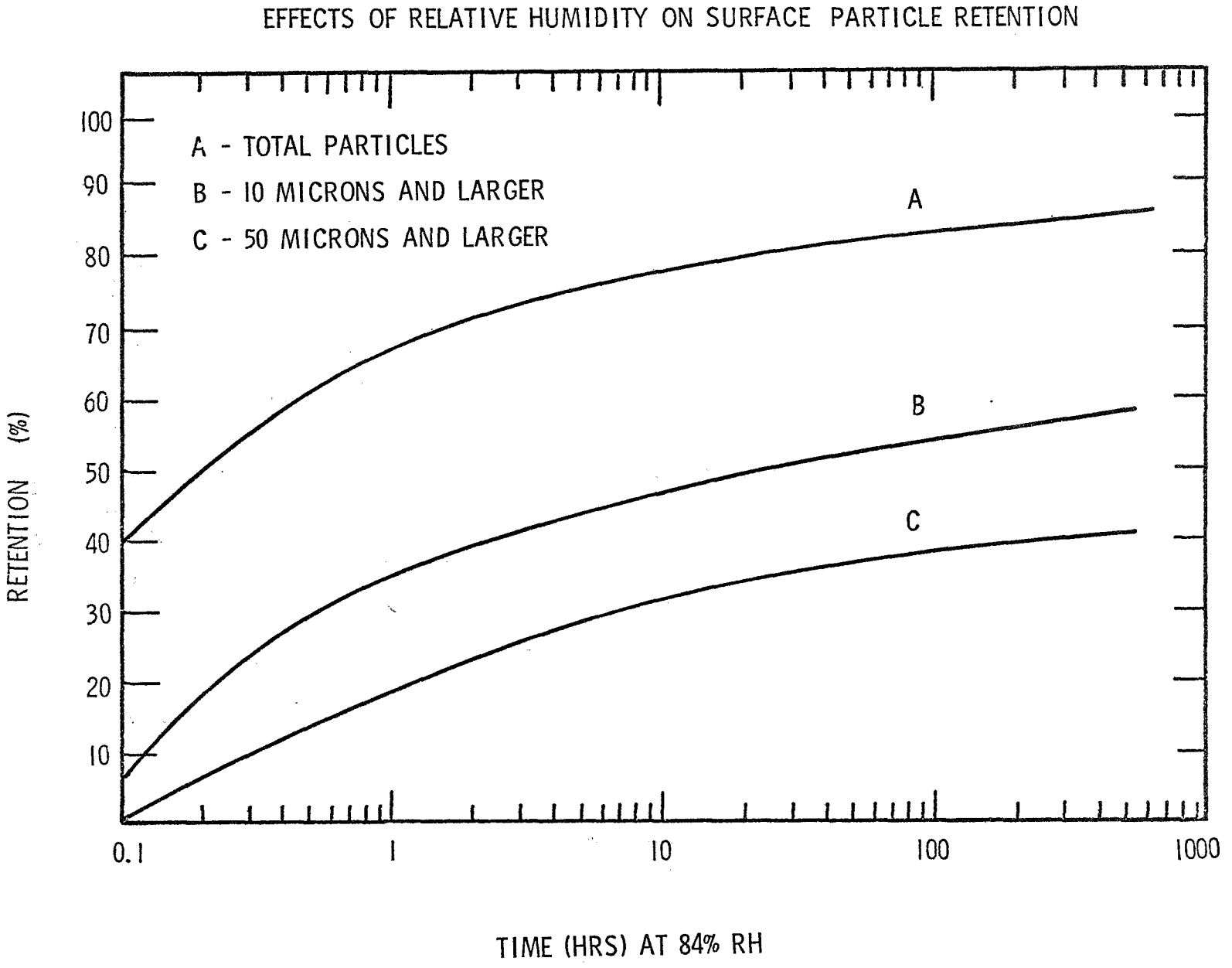

EFFECTS OF RELATIVE HUMIDITY ON SURFACE PARTICLE RETENTION
A - TOTAL PARTICLES
B - 10 MICRONS AND LARGER
C - 50 MICRONS AND LARGER
A
B
C
RETENTION (%)
100
90
80
70
60
50
40
30
20
10
0.1
1
10
100
1000
TIME (HRS) AT 84% RH

Figure 13

EFFECTS OF RELATIVE HUMIDITY ON SURFACE PARTICLE RETENTION

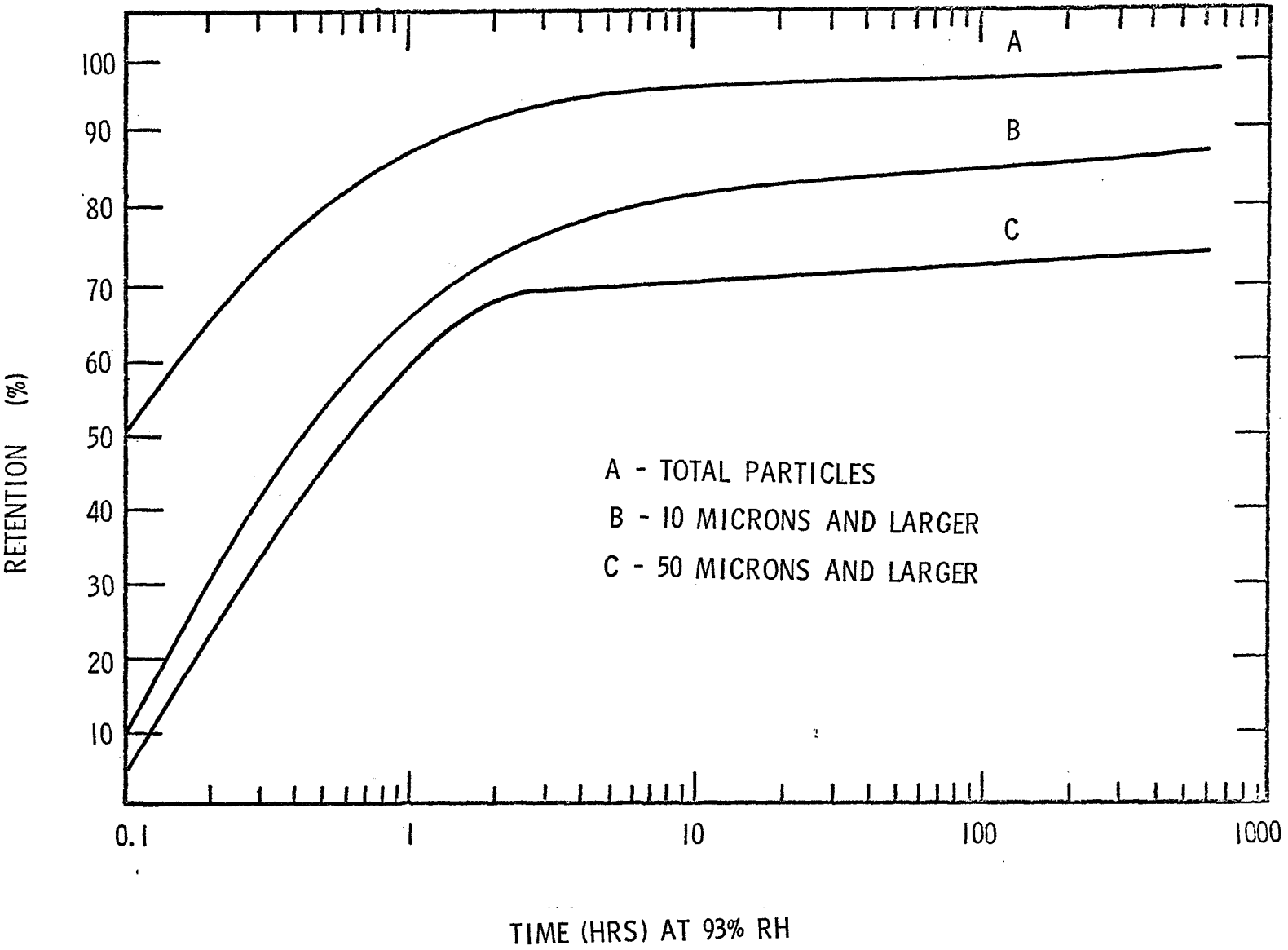


Figure 14

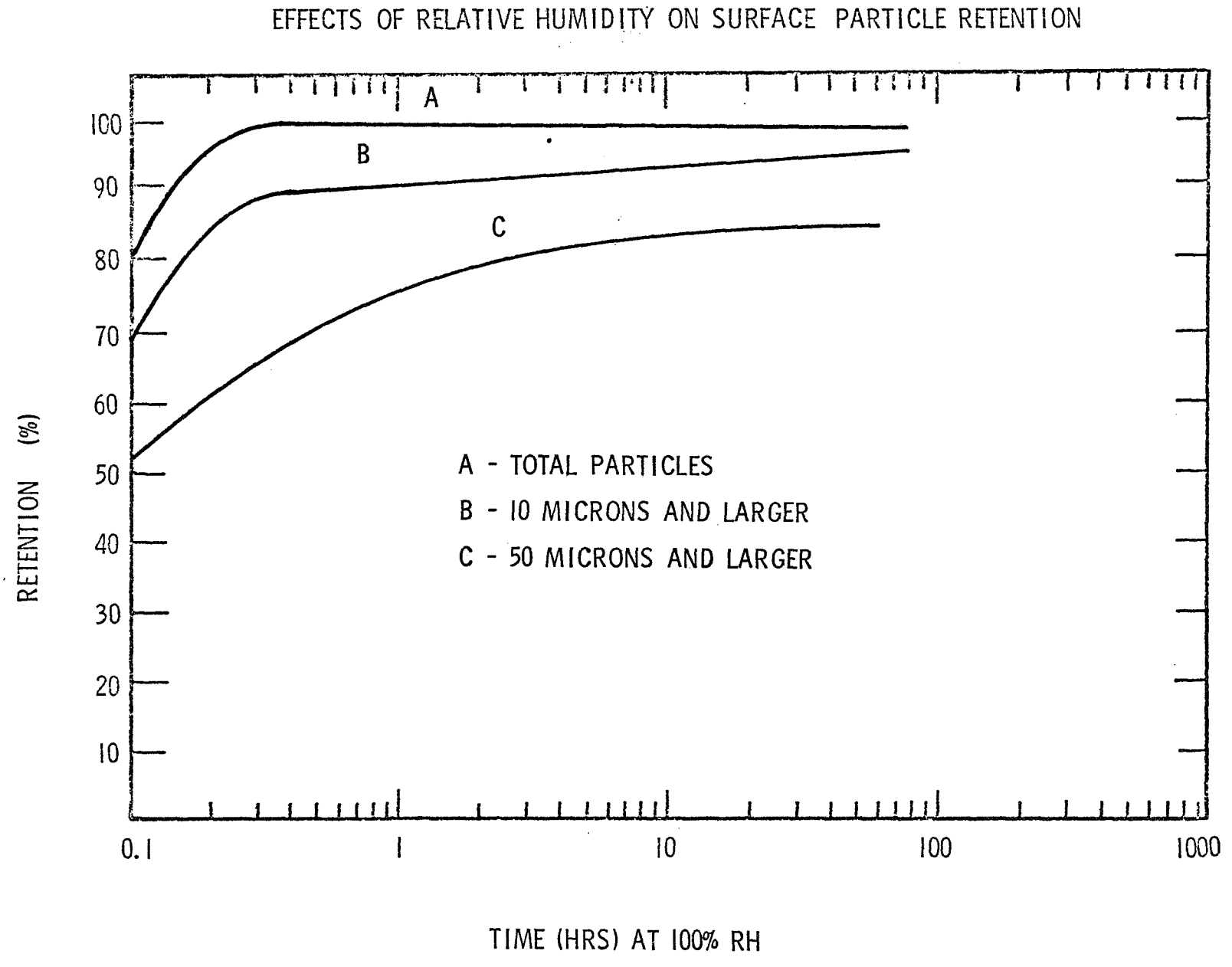

EFFECTS OF RELATIVE HUMIDITY ON SURFACE PARTICLE RETENTION
RETENTION (%)
100
90
80
70
60
50
40
30
20
10
A
B
C
A - TOTAL PARTICLES
B - 10 MICRONS AND LARGER
C - 50 MICRONS AND LARGER
0.1
1
10
100
1000
TIME (HRS) AT 100% RH

Figure 15

EFFECTS OF RELATIVE HUMIDITY ON SURFACE PARTICLE RETENTION

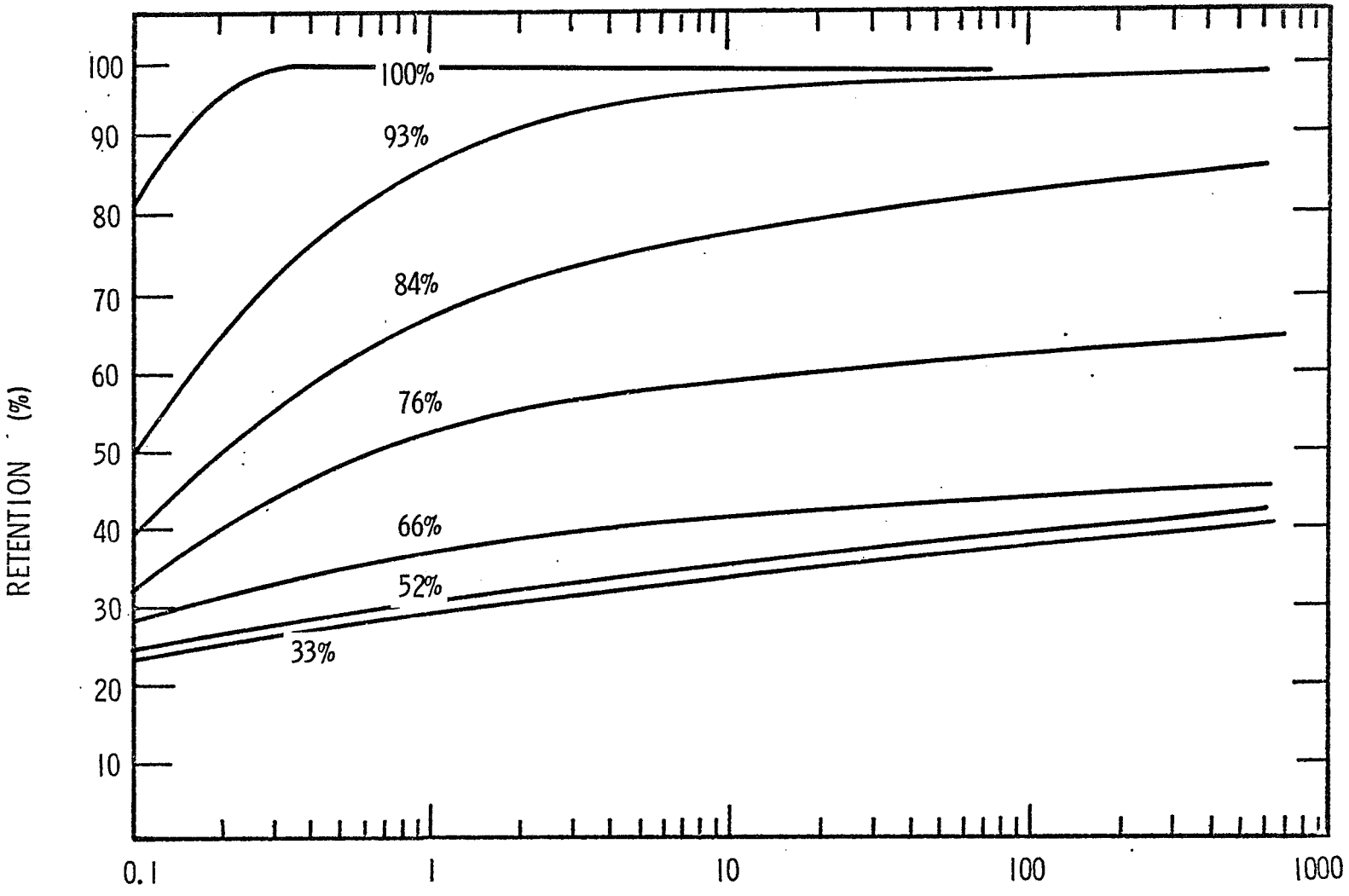


Figure 16

Studies on Bacterial Spore Inactivation

A. Description. A study demonstrating that the germination of bacterial spores can be inhibited by low concentrations of either aliphatic or aromatic alcohols was described in QR-19 (also Appl. Micobiol. 20, 620-623). An interesting feature of this inhibition is that it is completely reversible. Spore germination and subsequent spore outgrowth proceed normally when spores are removed from a germinating media containing alcohol, via Millipore filtration or centrifugation, and resuspended in a germinating media free of alcohol. Thus, the effect of alcohols on spore development appears to be sporostatic and not sporocidal.

Speculation as to the mechanism for the inhibition of germination by alcohols must account for two observations: (1) the low levels of alcohol required for complete inhibition, and (2) the reversibility of the inhibition. Such considerations strongly suggest that the alcohols are functioning by inhibiting enzyme(s) required for germination. If this postulate is valid it should be possible to obtain a chemically reactive structural analog of the alcohols which would combine irreversibly with the proposed spore enzyme(s) required for spore germination. The inactivation of the germinating enzyme(s) by a chemically reactive alcohol analog should then result in spore inactivation as determined by colony forming capacity. Part of this quarter's research efforts were directed towards investigating the effects of aqueous formaldehyde on the germination properties of B. subtilis var. niger spores.

B. Progress. The process of spore germination can be followed by observing the changes in optical density for a spore suspension as a function of time. Figure 17 illustrates the data obtained when spores of B. subtilis var. niger were exposed to a germinating media (4% Trypticase Soy Broth) in the absence and presence of aqueous formaldehyde at 30°C. Increasing the formaldehyde concentration in the germinating media caused a decrease in the extent of spore germination. This experimental result is equivalent to that obtained with alcohols, i.e., increasing alcohol concentration in the germinating media caused a decrease in the extent of spore germination. A plot of the extent of germination as a function of formaldehyde concentration is presented in Figure 18. From such data it was possible to obtain an extrapolated value of approximately 1.4% as the level of aqueous formaldehyde required to completely inhibit B. subtilis var. niger spore germination.

The spore suspensions containing the various formaldehyde concentrations (see Figure 17) were serially diluted and plated on Trypticase Soy Agar. The colony counts obtained from the spore suspensions exposed to aqueous formaldehyde were nearly a log lower than the colony counts obtained from the control spore suspensions not exposed to formaldehyde. This decrease of spore viability on exposure to aqueous formaldehyde suggests that it exhibits both sporostatic and sporocidal properties since it can produce decreased survival levels (sporocidal property), and inhibit spore germination which is partially reversible with serial dilutions (suggesting a sporostatic property). Alcohols, on the other hand, were observed to be solely sporostatic in action since their effect on spore germination was totally reversible.

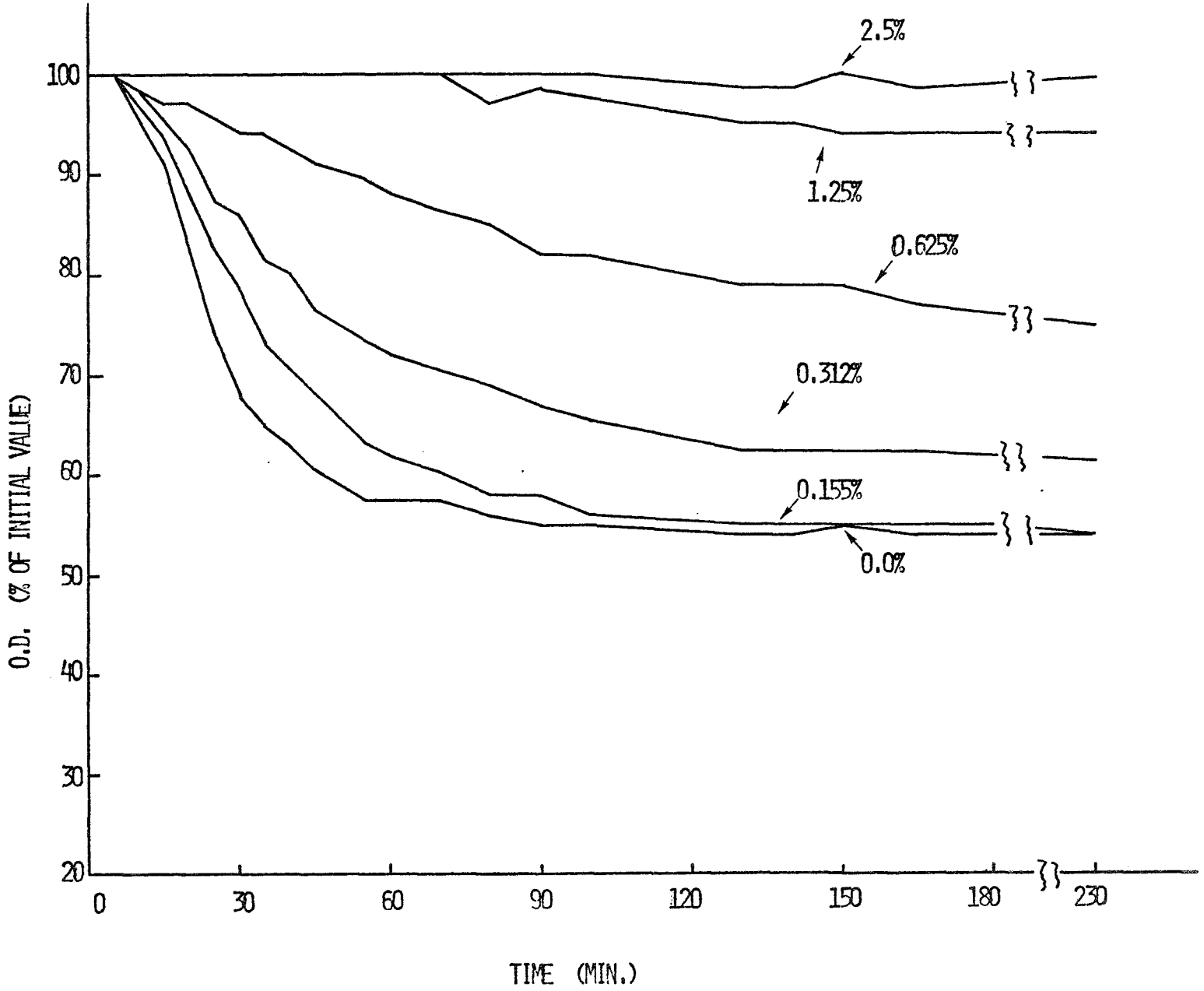

2.5%
1.25%
0.625%
0.312%
0.155%
0.0%
O.D. (% OF INITIAL VALUE)
100
90
80
70
60
50
40
30
20
0
30
60
90
120
150
180
230
TIME (MIN.)

Figure 17

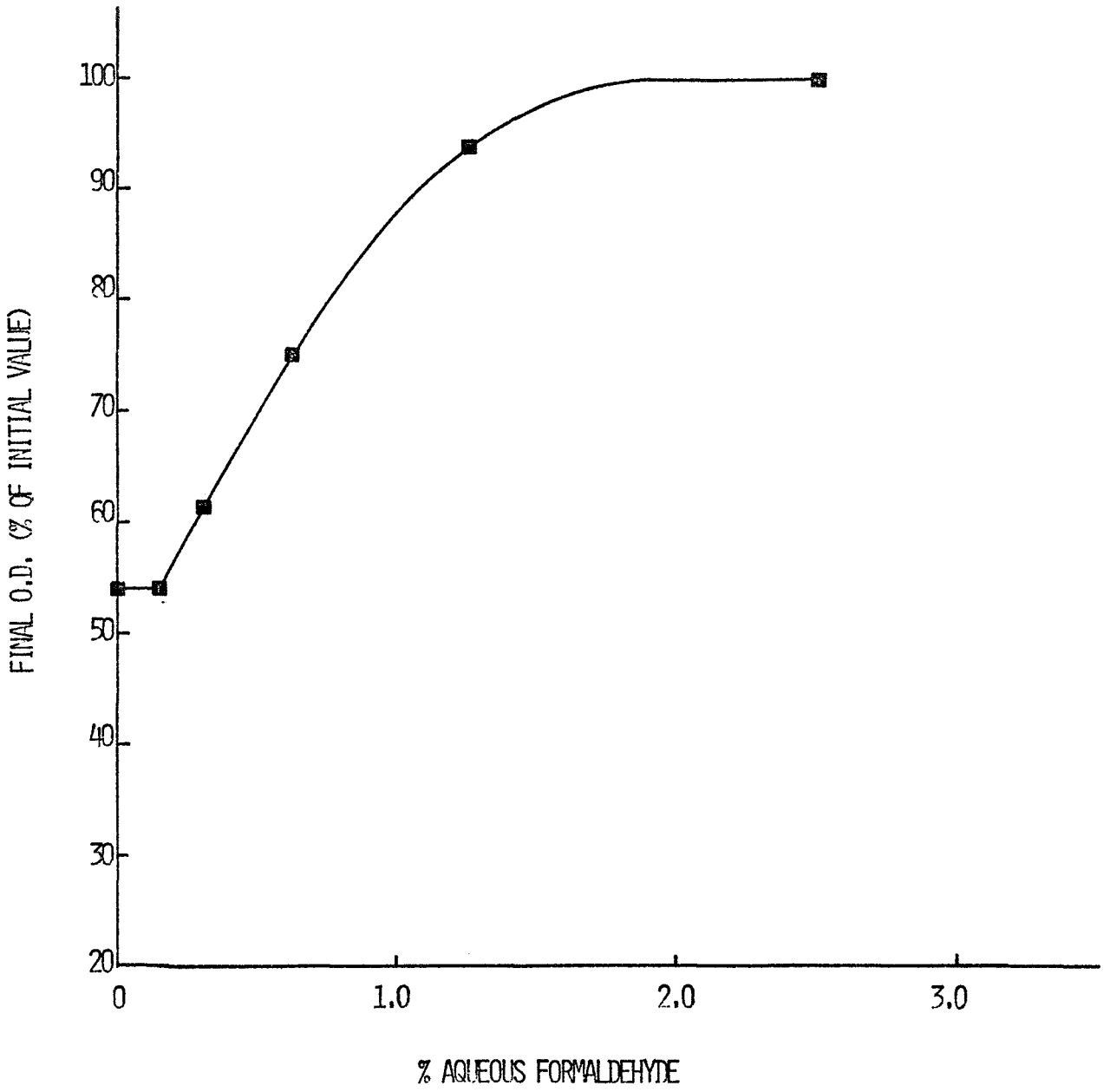

FINAL O.D. (% OF INITIAL VALUE)
100
90
80
70
60
50
40
30
20
0
1.0
2.0
3.0
% AQUEOUS FORMALDEHYDE

Figure 18

Since there seemed to be parallels between the effect of alcohols and formaldehyde on spore germination it was decided to investigate to what extent the inhibition of spore germination produced by formaldehyde was reversible for comparison with the known reversible inhibition of spore germination by alcohols. Figure 19 illustrates the reversibility of the inhibition of spore germination by aqueous formaldehyde. B. subtilis spores were suspended in Trypticase Soy Broth containing (A) no added formaldehyde and (B) a 2.5% formaldehyde solution. After one hour, spore suspension B was passed through a membrane filter (Millipore Corporation) and resuspended in Trypticase Soy Broth containing (C) a 2.5% formaldehyde solution and (D) no added formaldehyde. The resuspended spores germinated in media containing no formaldehyde, while the spores resuspended in media containing formaldehyde did not germinate. Thus, the inhibition of spore germination by both aqueous formaldehyde and alcohol is reversible, so that the partial inactivation of the population by aqueous formaldehyde is not caused by germination inhibition.

This investigation into the effect of aqueous formaldehyde on the process of spore germination has established the similarity of action of alcohols and formaldehyde on spore germination, i.e., both reversibly inhibit spore germination. Structurally and chemically an aldehyde is different from an alcohol and yet both appear to affect the spore germination process in a similar manner. Insight as to why formaldehyde should act as an alcohol in its effect on spore germination can be obtained from basic chemical considerations. Formaldehyde gas

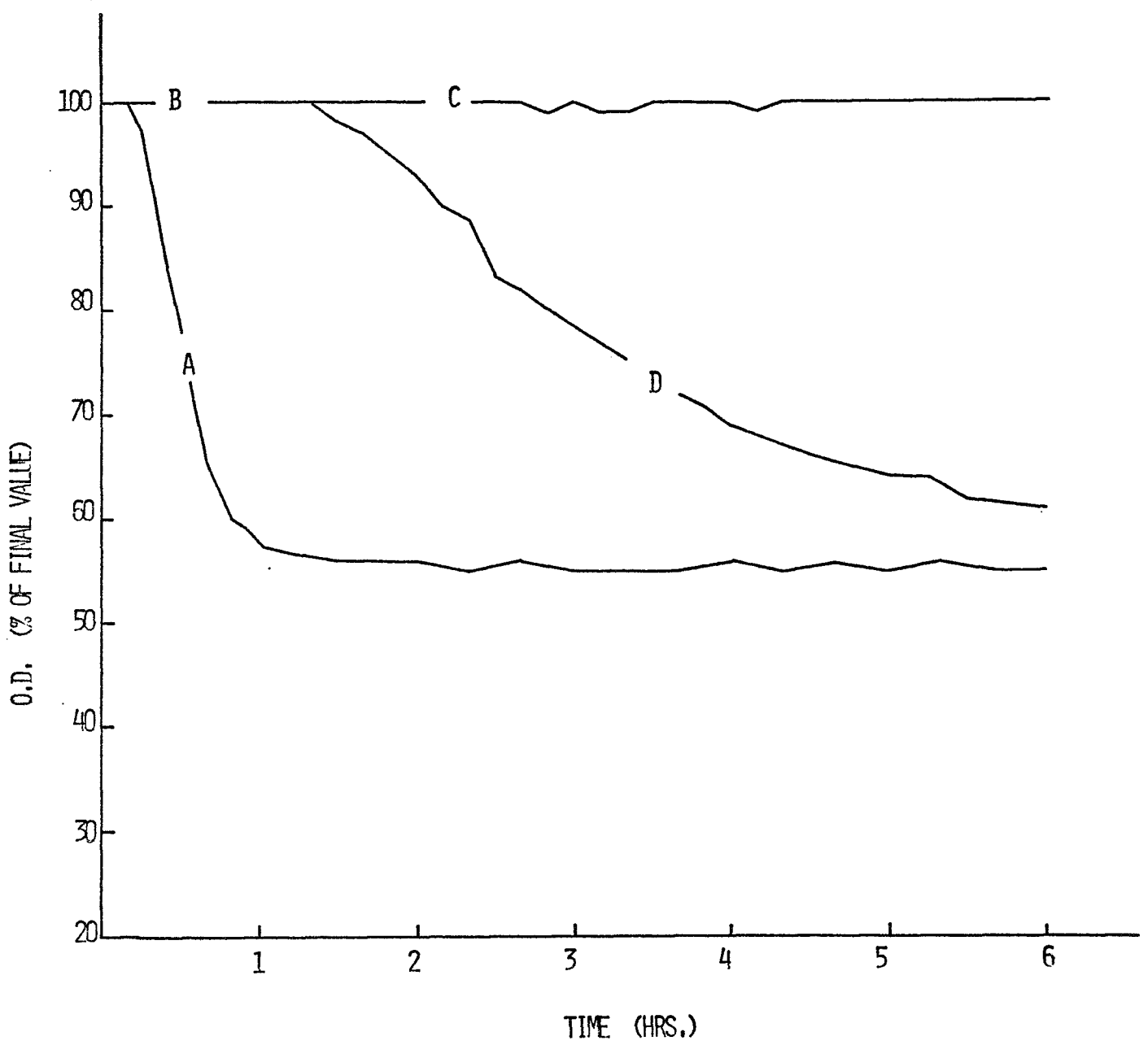
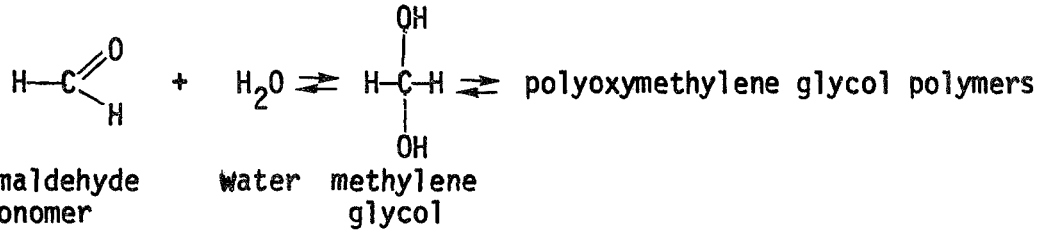

100
90
80
70
60
50
40
30
20
B
C
A
D
O.D. (% OF FINAL VALUE)
1
2
3
4
5
6
TIME (HRS.)

Figure 19

on dissolving in water rapidly reacts with water to form a monohydrate, methylene glycol, $CH_2(OH)_2$, and a series of low molecular weight polymeric or polyoxyethylene glycols, having the type formula, $HO(CH_2O)_nH$. The reaction of formaldehyde with water is:



The methylene glycol combines with other molecules of methylene glycol to yield polymers of polymethylene glycol of varying molecular weight. It has been determined that at 30°C a 2% formaldehyde solution contains 0.001% formaldehyde monomer and 99.999% methylene glycol and polymers of methylene glycol. Formaldehyde in an aqueous medium is 99.999% in the form of a di-alcohol (methylene glycol) or polymeric di-alcohols. Therefore, formaldehyde acts like an alcohol in inhibiting spore germination because in an aqueous solution formaldehyde exists essentially as a di-alcohol. That di-alcohols *per se* can inhibit spore germination was shown in a study in which ethylene glycol reversibly inhibited the germination of *B. subtilis* var. *niger* spores.

Table 1 compares physical and sporostatic properties of aqueous formaldehyde (methylene glycol) and ethanol. Calculations based on the data in Table 1 reveal that nearly identical amounts of ethanol and methylene glycol are required to inhibit a single *B. subtilis* var. *niger* spore.

Table 1

Additive	Structure	Mol. Wt.	Germination Inhibiting Concentration	Column A*
Ethanol	CH_3-CH_2-OH	46.07	1.4%	1.46×10^{14}
Methylene Glycol	HO-CH_2-OH	48.03	1.4%	1.75×10^{14}

This result suggests that these additives, of comparable molecular weight, steric volume and chemical substituents, are interacting with the spore in the same manner to inhibit spore germination; perhaps, by combining with spore enzyme(s) required for spore germination. The observation that aqueous formaldehyde exhibits some sporocidal activity and that the rate of the reversal of spore inhibition by aqueous formaldehyde is approximately five times slower than that observed for ethanol suggests that while both additives may be interacting with the same spore component, with the same stoichiometry, they do so in a different manner.

*Column A = the figures in Column A refer to the number of atoms of additive required to inhibit one B. subtilis var. niger spore.

Statistical Analysis of Experimental Data

A. Description. In many of our experimental programs, it is often desirable to fit data collected on the destruction pattern of microorganisms to the log model of bacterial inactivation. The development of a computer program was undertaken to facilitate the processing of the hundreds of plate counts which are collected during the course of a series of experiments. The properties of this program were to be:

(1) The program should handle replicate plates and more than one dilution for each sampling time period.

(2) The best dilution at each sampling period should be chosen on the basis of the tightness of the data.

(3) The D-value should be computed.

(4) The initial population should be treated as only an additional data point and no special significance assigned to it. Thus, the theoretical intercept should be computed.

B. Progress. A computer program with the properties outlined above has been developed. The documentation is now in the process of being completed. When this is completed the program will be made available to other experimental groups. A flow chart for this program is given in Figure 20. We shall briefly discuss some of the statistical techniques used. Let

$x_{ij}(t_\ell)$ = number of colonies on plate i of dilution j at sampling period ℓ,

$\ell = 1, \ldots, M$

$j = 1, \ldots, K_\ell$

$i = 1, \ldots, N_{j\ell}$

where

M = number of sampling periods

K_ℓ = number of dilutions at sampling period ℓ

$N_{j\ell}$ = number of plates of dilution j for sampling period ℓ

and

t_ℓ = time of sampling period ℓ (in any units desired).

The mean of the plate counts for a particular dilution (j) and sampling period (t_ℓ) is

$$\bar{x}_j(t_\ell) = \frac{1}{N_{j\ell}} \sum_{i=1}^{N_{j\ell}} x_{ij}(t_\ell)$$

while the variance and standard deviation are defined to be

$$s_j^2(t_\ell) = \frac{\sum_{i=1}^{N_{j\ell}} \left(\bar{x}_j(t_\ell) - x_{ij}(t_\ell) \right)^2}{N_{j\ell} - 1}$$

$$= \frac{\sum_{i=1}^{N_{j\ell}} \left(x_{ij}(t_\ell) \right)^2 - N_{j\ell}\, \bar{x}_j(t_\ell)}{N_{j\ell} - 1}$$

and

$$s_j(t_\ell) = \sqrt{s_j^2(t_\ell)}$$

respectively.

Another desirable quantity for each dilution at each time period is a confidence interval for the mean. The 95% confidence level for a particular dilution at a particular time, t_ℓ, is approximated by

$$\left[\bar{x}_j(t_\ell) - \frac{k\, s_j(t_\ell)}{\sqrt{N_{j\ell}}}, \quad \bar{x}_j(t_\ell) + \frac{k\, s_j(t_\ell)}{\sqrt{N_{j\ell}}} \right]$$

where k is chosen from the students t-distribution with $N_{j\ell} - 1$ degrees of freedom by

$$k = 1.96 \left\{ 1 + \frac{(1.96)^2 + 1}{2(N_{j\ell} - 1)} + \frac{[(1.96)^2 + 3]\,[5(1.96)^2 + 1]}{96(N_{j\ell} - 1)^2} \right\}$$

A good measure of the amount of spread in a particular set of data has been found to be the relative standard deviation. This is more commonly known as the coefficient of variation. For each sampling period (t_ℓ) and each dilution (j) it is defined to be

$$C_j(t_\ell) = \frac{s_j(t_\ell)}{\bar{x}_j(t_\ell)} \quad .$$

In calculating the fit to the data of the log model, we wish to use the dilution at each time period which has the "tightest" data. The coefficient of variation is used as an index of this spread. Therefore, we let

$$X(t_\ell) = \bar{x}_J(t_\ell)$$

where J is chosen to minimize $C_j(t_\ell)$ for $j = 1, \ldots, K_\ell$. Let the degree of this dilution be d_ℓ.

We can now prepare to calculate the parameters in our log model for the conditions of the experiment under consideration. For purposes here, the log model is assumed to take the form

$$E(N(t)) = E(N(0))\, 10^{-t/D}$$

where N(t) is the number of organisms at time t (≥ 0). If we let

$$Y(t) = \log_e [E(N(t))],$$

then Y(t) takes the form

$$Y(t) = \alpha + \beta t$$

where, in theory, $\alpha = \log_e [E(N(0))]$ and

$$\beta = \frac{-\log_e 10}{D} \quad .$$

The parameters that we will initially estimate will be α and β. From these, $e^{est\ (\alpha)}$ will give an estimate of E(N(0)), and an estimation of D will be

$$\frac{-\log_e 10}{est\ (\beta)} \quad .$$

To this end, we let

$$Y(t_\ell) = \log \left[X(t_\ell) \times 10^{d_\ell + 1} \right] \quad ,$$

$$\bar{t} = \frac{\sum_{\ell=1}^{M} t_\ell}{M}$$

and

$$\bar{Y} = \frac{\sum_{\ell=1}^{M} Y(t_\ell)}{M} \quad .$$

Then we shall approximate α and β by a and b, where

$$b = \frac{\sum_{\ell=1}^{M} (t_\ell - \bar{t})(Y(t_\ell) - \bar{Y})}{\sum_{\ell=1}^{M} (t_\ell - \bar{t})^2} = \frac{\sum_{\ell=1}^{M} (t_\ell - \bar{t})\, Y(t_\ell)}{\sum_{\ell=1}^{M} (t_\ell - \bar{t})^2}$$

and

$$a = \bar{Y} - b\bar{t}$$

respectively. We know from the Gauss-Markoff theorem that the expected values of a and b satisfy $E(a) = \alpha$ and $E(b) = \beta$. Therefore the line

$$Z = a + bt$$

provides a "fit" as described above to the selected means $Y(t_\ell)$ at the sampling periods t_ℓ. Having a and b permits estimation of

$$E(N(0)) \text{ by } e^a \qquad \text{and of}$$

$$D \quad \text{by} \quad \frac{-\log_e 10}{b} \quad .$$

It is desirable in many applications to have a measure of how nearly the variation among the $Y(t_\ell)$ may be explained solely on the basis of time. The correlation coefficient between $Y(t_\ell)$'s and time, denoted by r, performs this duty. This is defined by

$$r = \frac{\sum_{\ell=1}^{M} (t_\ell - \bar{t})(Y(t_\ell) - \bar{Y})}{\sqrt{\sum_{\ell=1}^{M} (t_\ell - \bar{t})^2 \sum_{\ell=1}^{M} (Y(t_\ell) - \bar{Y})^2}} = \frac{\sum_{\ell=1}^{M} t_\ell (Y(t_\ell))}{\sqrt{\sum_{\ell=1}^{M} t_\ell^2 \sum_{\ell=1}^{M} Y(t_\ell)^2}}$$

We observe that $-1 \leq r \leq 1$. If r is near +1 or -1 then the one variable, time, is capable of "explaining" the variation between the $Y(t_\ell)$ whereas, if r is near 0, neither variable explains the variation in the other.

There are several errors about which it is often convenient to have some information. The standard error in the estimated slope (b) is given by

$$S_b = \frac{S_{Yt}}{\sqrt{\sum_{\ell=1}^{M} (t_\ell - \bar{t})^2}}$$

and the standard error of estimate of Y from t, S_{Yt}, is estimated by

$$S_{Yt} = \sqrt{\frac{\sum_{\ell=1}^{M} (Z(t_\ell) - Y(t_\ell))^2}{M - 2}} \quad .$$

The standard error in the estimated log intercept (a) is given by

$$S_a = S_{Yt} \left[\frac{1}{M} + \frac{t^{-2}}{\sum_{\ell=1}^{M} (t_\ell - \bar{t})^2} \right]^{1/2}$$

Another feature which it is sometimes desirable to have available which occurs in the program is the confidence band about the calculated line. To compute this, let

$$S_{Z(t_\ell)} = S_{Yt} \sqrt{\frac{1}{M} + \frac{t_\ell - \bar{t}}{\sum_{\ell=1}^{M} (t_\ell - \bar{t})^2}} \quad .$$

Then, the upper 95% confidence level is given by

$$Z_u(t_\ell) = a + b\, t_\ell + k\, S_{Z(t_\ell)}$$

and the lower by

$$Z_L(t_\ell) = a + b\, t_\ell - k\, S_{Z(t_\ell)}$$

where k is defined from the students t-distribution with M-2 degrees of freedom.

An example of the output from this program is given in Figure 21.

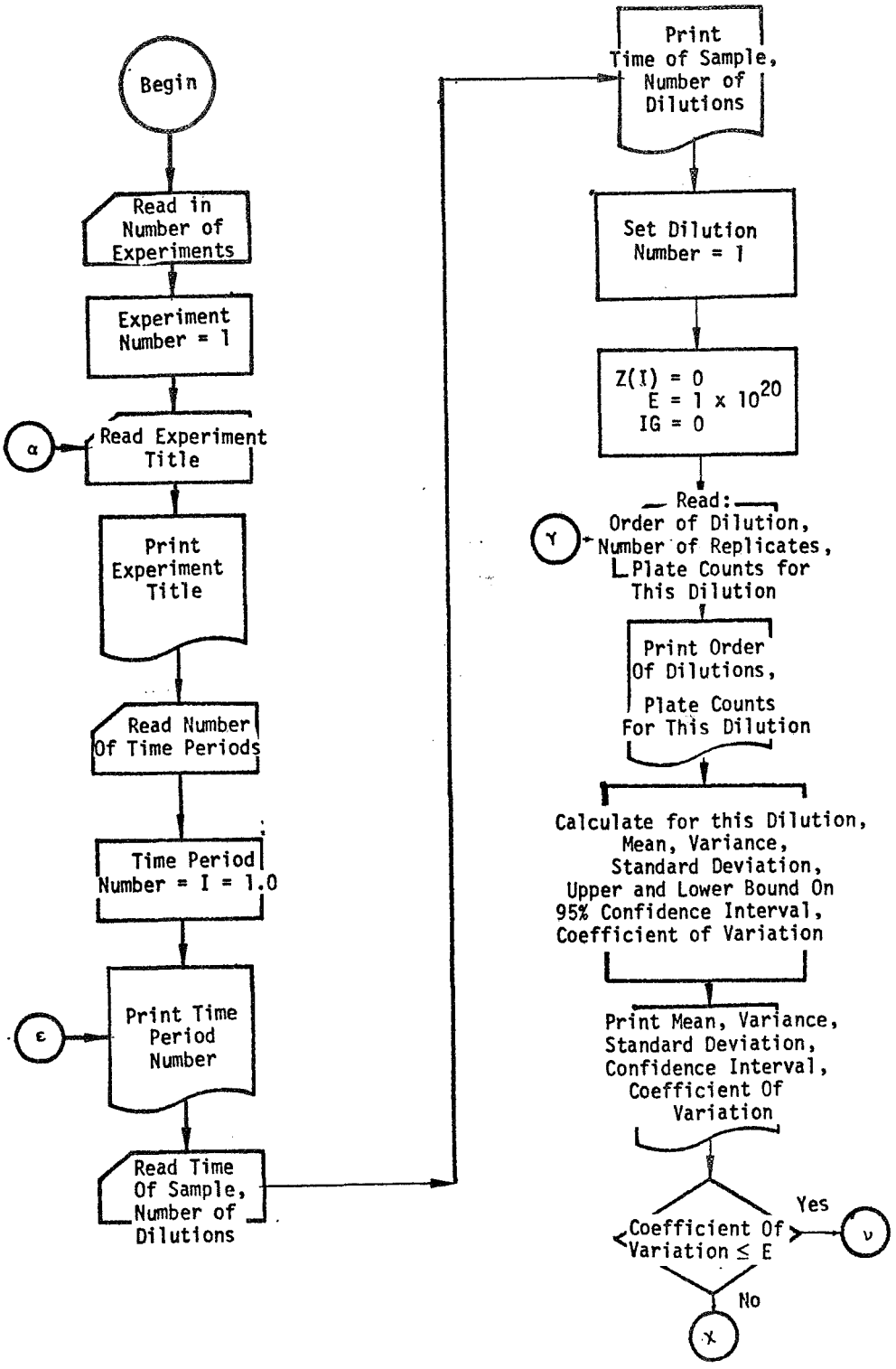

Begin
Read in Number of Experiments
Experiment Number = 1
α
Read Experiment Title
Print Experiment Title
Read Number Of Time Periods
Time Period Number = I = 1.0
ε
Print Time Period Number
Read Time Of Sample, Number of Dilutions
Print Time of Sample, Number of Dilutions
Set Dilution Number = 1
Z(I) = 0
E = 1 x 10^20
IG = 0
γ
Read: Order of Dilution, Number of Replicates, Plate Counts for This Dilution
Print Order Of Dilutions, Plate Counts For This Dilution
Calculate for this Dilution, Mean, Variance, Standard Deviation, Upper and Lower Bound On 95% Confidence Interval, Coefficient of Variation
Print Mean, Variance, Standard Deviation, Confidence Interval, Coefficient Of Variation
Coefficient Of Variation ≤ E
Yes
ν
No
χ

Figure 20

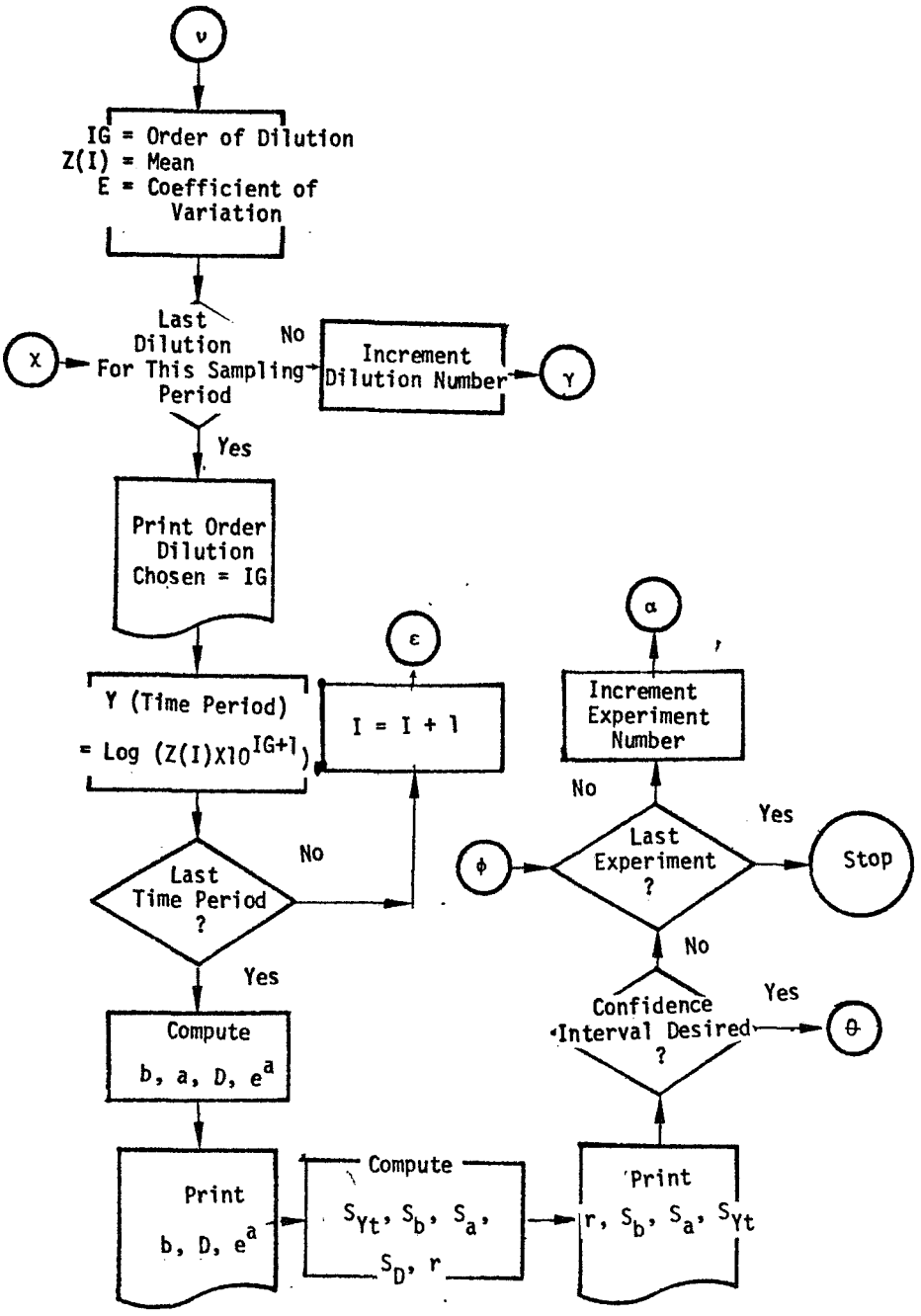


Figure 20 (Continued)

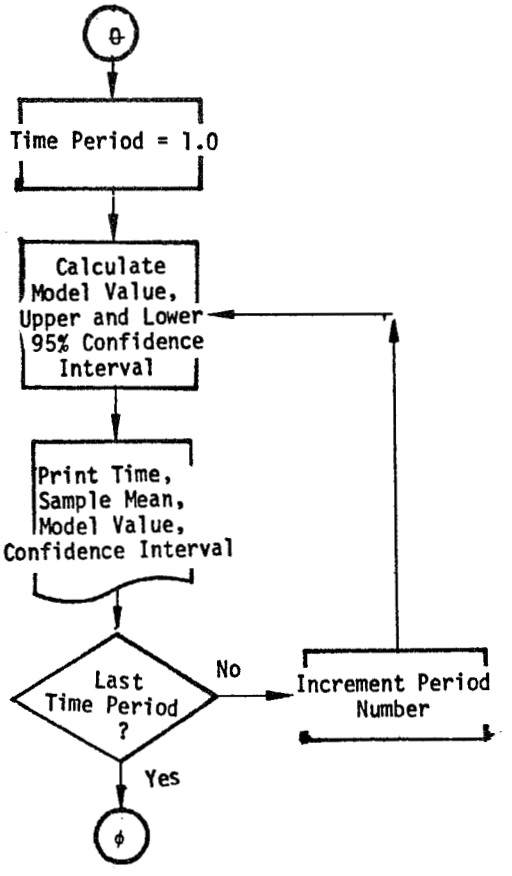


Figure 20 (Continued)

```
 DATA SET =    1
TIME=    0.000
 NO. DIL.=    1
NUMBER DATA POINTS=    8
 ORDER OF DIL. =    3
DATA
  265.00   282.00   292.00   267.00   250.00   277.00   265.00   285.00
MEAN= 272.875  VARIANCE=   185.0  S.D.=    13.6  UPPER .95 C.I.=   284.2  LOWER .95 C.I.=   261.5  CV =   .0498
 CIL. CHOSEN =    3

 DATA SET =    2
TIME=    3.000
 NO. DIL.=    1
NUMBER DATA POINTS=    8
 ORDER OF DIL. =    3
DATA
   67.00    58.00    62.00    57.00    51.00    75.00    80.00    72.00
MEAN=  65.250  VARIANCE=    99.4  S.D.=    10.0  UPPER .95 C.I.=    73.6  LOWER .95 C.I.=    56.9  CV =   .1528
 CIL. CHOSEN =    3

 DATA SET =    3
TIME=    6.000
 NO. DIL.=    1
NUMBER DATA POINTS=    8
 ORDER OF DIL. =    2
DATA
   58.00    63.00    80.00    83.00    62.00    66.00    75.00    63.00
MEAN=  69.750  VARIANCE=    86.2  S.D.=     9.3  UPPER .95 C.I.=    76.5  LOWER .95 C.I.=    61.0  CV =   .1351
 DIL. CHOSEN =    2

 DATA SET =    4
TIME=    9.000
 NO. DIL.=    1
NUMBER DATA POINTS=    8
 ORDER OF DIL. =    1
DATA
  278.00   212.00   197.00   201.00   214.00   255.00   236.00   231.00
MEAN= 228.000  VARIANCE=   777.7  S.D.=    27.9  UPPER .95 C.I.=   251.2  LOWER .95 C.I.=   204.8  CV =   .1223
 CIL. CHOSEN =    1

 DATA SET =    5
TIME=   12.000
 NO. DIL.=    1
NUMBER DATA POINTS=    8
 ORDER OF DIL. =    1
DATA
   48.00    37.00    37.00    25.00    25.00    21.00    42.00    29.00
MEAN=  33.000  VARIANCE=    89.4  S.D.=     9.5  UPPER .95 C.I.=    40.9  LOWER .95 C.I.=    25.1  CV =   .2866
 CIL. CHOSEN =    1
```

Figure 21

```
 DATA SET =     6
TIME=  15.000
 NO. DIL.=     1
NUMBER DATA POINTS=     8
 ORDER OF DIL. =     0
DATA
  116.00   135.00    87.00    62.00    95.00    82.00    85.00    84.00
MEAN=  93.250  VARIANCE=   508.5  S.D.=    22.5  UPPER .95 C.I.=   112.0  LOWER .95 C.I.=    74.5  CV =   .2418
 CIL. CHOSEN =     0

 DATA SET =     7
TIME=  18.000
 NO. DIL.=     2
NUMBER DATA POINTS=     8
 ORDER OF DIL. =     0
DATA
   26.00    16.00    12.00    14.00    11.00    10.00    18.00     9.00
MEAN=  14.500  VARIANCE=    30.9  S.D.=     5.6  UPPER .95 C.I.=    19.1  LOWER .95 C.I.=     9.9  CV =   .3831
NUMBER DATA POINTS=     4
 ORDER OF DIL. =    -1
DATA
  157.00   117.00    92.00   162.00
MEAN= 132.000  VARIANCE=  1116.7  S.D.=    33.4  UPPER .95 C.I.=   183.2  LOWER .95 C.I.=    80.8  CV =   .2532
 CIL. CHOSEN =    -1

 DATA SET =     8
TIME=  21.000
 NO. DIL.=     1
NUMBER DATA POINTS=     4
 ORDER OF DIL. =    -1
DATA
   51.00    50.00    93.00    89.00
MEAN=  70.750  VARIANCE=   549.6  S.D.=    23.4  UPPER .95 C.I.=   106.7  LOWER .95 C.I.=    34.8  CV =   .3314
 CIL. CHOSEN =    -1


SLOPE=    -.521       D VALUE=    4.421       INTERCEPT=     2.3104308639E+06
CORR. COEF.=     .58018 STAND. ERR. IN EST. SLOPE=     .01896 STAND. ERR. OF EST.=     .13595 STAND. ERR. IN EST. INTER.=
                                             .95 CONF. INTERVAL
         T                      SAMP                MODEL                UPPER                LOWER
    0.                    2.7287500000E+06    2.3104308639E+06    4.1234704598E+06    1.2945626333E+06
    3.0000000000E+00      6.5250000000E+05    4.8409570870E+05    7.7422063309E+05    3.0268975685E+05
    6.0000000000E+00      6.8750000000E+04    1.0143071530E+05    1.4820424844E+05    6.9418995170E+04
    9.0000000000E+00      2.2800000000E+04    2.1252388364E+04    2.9406330336E+04    1.5359414317E+04
    1.2000000000E+01      3.3000000000E+03    4.4529313417E+03    6.1613954984E+03    3.2181991139E+03
    1.5000000000E+01      9.3250000000E+02    9.3300560831E+02    1.3632497272E+03    6.3854732390E+02
    1.8000000000E+01      1.3200000000E+02    1.9548908311E+02    3.1264826143E+02    1.2223314929E+02
    2.1000000000E+01      7.0750000000E+01    4.0960077062E+01    7.3102238390E+01    2.2950431476E+01
```

Figure 21 (Continued)

Computerized Identification System

A. Description. The objective of this activity has been the development of a computerized version of the PHS microbiological identification system in use at the Cape, so that such a program could be incorporated in the Lunar Information System. This would relieve the PHS of the actual identification of the colonies sampled during the outbound contamination inventory of Apollo spacecraft.

B. Progress.

BUGID. Feasibility of modeling the identification system has been previously reported in QR-15 and QR-17. A completed version of the program, called BUGID, has been installed in the Lunar Information System in use at the Cape. It is incorporated in the program QUAL and has been used to process and identify Apollo 14 data. For this first actual use (Apollo 14), PHS personnel at the Cape made an identification and compared results with that of the program. They were satisfied and will use the computerized version in the future. Thus, except for a few additional results gained during this quarter, reported below, this activity is complete.

Unspecified Microorganisms. During the process of simulating and checking the identification system in use, a curiosity in the data was observed. An exceedingly ideal identification system using good reliable tests for discriminating would identify an unknown with all test results agreeing with that specified for the appropriate organism every time. However, at present, biological variation and the state

of identification systems result in less precise results; sometimes because of variation in laboratory test results and also because the identification schemes themselves have expected test results listed as variable or weak or unknown. It will be recalled that the PHS established some guidelines, as rules, to cope with this variation. These were that an identification could be made if there were not more than two tests on an unknown which disagreed with those results specified by the identification system and then, when there were two, only a single organism category was to be involved. In the case where one test result for an unknown was not as indicated for an organism category in the scheme an identification could be made if not more than three organism categories were indicated, i.e., a choice must be made between three or fewer organism categories when one test result (possibly different for each of the three of fewer categories) was different from that specified by the scheme. Of course, an identification can clearly be made when exact agreement is obtained between test results for an unknown and the results specified in the scheme for a single organism category. These rules were incorporated into the program along with a simulation of the choice among multiple possible categories made by the PHS in the past.

During the checkout of the program with the existing Apollo 10, 11, 12 and 13 data, an interesting phenomenon was observed. In Scheme A--(Baird-Parker) for catalase positive, gram positive cocci (containing some 70% of all Apollo samples) most of the identifications were made on the basis of an exact agreement between the data observed and the test outcomes prescribed by the scheme. As the number of categories among which a choice had to be made or the number of observed

test results differing from those specified by the scheme increased, the frequency with which that event occurred decreased. This could be regarded as a measure of the effectiveness of the scheme indicating that it was quite good. There were, however, some exceptions to the pattern. One in particular stood out in category number 7 (Micrococcus, subgroup 1) where the most common mode of identification was as a choice among three categories, each of which had one prescribed test result which did not agree with the observed data. Upon examination it developed that, in every case, the three categories were Nos. 7, 13 and 14, which are Baird-Parker's Micrococcus (Cohn) subgroups 1, 7 and 8. An analysis of Baird-Parker's key indicated that there was only one set of test results which would disagree with the key at these categories in one test result (SC-RR-70-779) so it was not too great a surprise to learn that the same test results had been recorded for these samples.

Thus, one of the organisms more commonly observed on Apollo spacecraft is a gram positive, catalase positive cocci which does not fit any of the Baird-Parker subgroups or classifications. It is the third most commonly seen species and represents 5% of the total Apollo samples. A check of the current edition of Bergey's Manual reveal that this frequently occurring unknown is not listed there either. The observed test sequence for this unknown differs from those 16 species listed in the genus Micrococcus by pigmentation, acid from glucose (aerobic), acid from lactose or acid from maltose.

Thus, it would appear that some 5% of the microorganisms recovered from Apollo spacecraft by the PHS have not been previously precisely

specified either in Bergey's or by the Baird-Parker scheme presently in use. This is curious, in view of their apparent prevalance in a natural environment. A joint paper with the PHS describing this finding is being prepared.

GENID. Because of continual improvement as the result of increased knowledge and experience, identification schemes are frequently modified or even changed entirely. As a result, the program BUGID for simulation of the PHS identification scheme was written so that almost all of the identification scheme was input to the program. Thus PHS personnel can easily modify the system as changes are desired. This abstract approach used in the development of BUGID was extended so that a very general program for bacterial identification, GENID, was written. For GENID, the entire identification system is input. This includes the schemes, the number of schemes, the tests used and any rules that might be used to resolve ambiguous cases.

GENID is a very simple program which essentially compares test results with those prescribed by the appropriate scheme (which is read as input). GENID proper consists of only 27 cards (including comment card) and four subroutines. These subroutines perform the functions indicated by their names: INPUT, SETUP, MATCH and RULES. SETUP and MATCH for GENID are very like the similarly named subroutines of BUGID. INPUT reads the identification system to be used and allocates the appropriate computer space for the system. RULES is the subroutine that applies the decision rules specified in the input to GENID to be applied to multiple identification situations.

Because of the extreme simplicity of GENID it is very fast in addition to being so flexible. Virtually any identification system can be simulated by the program. It is necessary to convert keys to matrix form if they are given in tree form. Since this can always be done there is no loss of generality through the use of matrices. As it is presently written, GENID prints the identification system at the beginning of the output for every run. This is tantamount to listing the input cards which contain the system. For example, that part of the present PHS system that is used for an immediate identification is as follows:

IDENTIFICATION MATRIX MOLDS ETC.

	90	91	88	89	-2
17	1	99	99	99	99
17	99	1	99	99	99
17	99	99	1	99	99
17	99	99	99	1	99

Columns 90, 91, 88, and 89 designate molds, yeasts, actinomyces, streptomyces. The last column is used to indicate the next step - here, proceed to the second (-2) identification matrix. The rules in the PHS system being used are listed as follows:

NUMBER OF TYPES OF DISAGREEMENTS = 3

NUMBER OF EACH TYPE ALLOWED = 1 3 1

Finally, in the program output, the data input card is listed, followed by the number of disagreements and the identification(s). Sample

Output is shown as Figure 22.

GENID is a very general program which may be used with almost any identification system without modification. It is not now planned to replace BUGID with GENID in the Lunar Information System, but GENID it available should any need arise. A report is being prepared.

Sample GENID Output

Tests Input on Sample Card

```
                                                                                      TYPE
INPUT CARD                                                                             OF   IDENTS.
                                                                                      DIS.
00002032770118111 2**2******************2***1131100*001*10*****01***********0371     0    37
00003032770118111 2**2******************2***1131100*001*10*****01***********0371     0    37
00004032770118212 2****************14331212*********2***********************0021     0     2
00005032770118111 2**2******************1***1131100*001*10*****01***********0371     0    37
00006032770118114 2**2******************2***1131100*001*10*****01***********0371     0    37
00007032770118111 2**2******************2***1131100*001*10*****01***********0371     0    37
00008032770118114 2**2******************2***1131100*001*10*****01***********0371     0    37
00009032770118114 2**2******************2***1131100*001*10*****01***********0371     0    37
00010032770118212 2****************11111211*********1***********************0131     0    13
00011032770118212 2****************13131211*********1***********************0071     1     7 13 14
00012032770118111 2**2******************2***1131100*001*10*****01***********0371     0    37
00013032770118111 2**2******************2***1131100*001*10*****01***********0371     0    37
00014032770118212 2****************13331211*********1***********************0081     1     8 11
00015032770118111 2**2******************2***1131100*001*10*****01***********0371     0    37
00016032770118212 2****************13111212*********1***********************0131     1     3 13
00017032770118114 2**2******************2***1131100*001*10*****01***********0371     0    37
00018032770118114 2**2******************2***1131100*001*1*******1***********0371     0    37
00020032770118114 2**2******************1***1131100*001*10*****0211*********0371     0    37
00021032770218114 2**2******************2***1131121*111*10*****0211*********0371     0    37
00022032770218212 2****************11111212*********1***********************0131     1    13
00023032770218212 2****************14331212*********2***********************0021     0     2
00024032770218212 2****************13131211*********1***********************0071     1     7 13 14
00025032770219212 2****************14113211*********1***********************0061     1     6
00026032770218212 2****************11113211*********2***********************0131               NO AGREEMENT
00027032770218114 2**2******************2***1131121***1*10*****0211*********0371     0    37
00028032770218212 2****************13131211*********1***********************0071     1     7 13 14
00029032770219212 2****************14113211*********1***********************0061     1     6
00030032770218212 2****************13131211*********1***********************0071     1     7 13 14
00032032770215212 2****************13131211*********1***********************0071     1     7 13 14
```

Figure 22

Federal Standard 209a

A. Description. Sandia Laboratories, through the AEC, has the delegated responsibility from the General Services Administration (GSA) to maintain Federal Standard 209a, "Clean Room and Work Station Requirements, Controlled Environment."

B. Progress. The proposed meeting of government representatives to review Federal Standard 209a and to determine whether a formal revision is needed (QR-20) will not be convened. Instead, we will proceed with plans for a revision and will attempt to carry out communications with members of the Working Group by mail. It may be necessary to call a meeting later if controversial issues emerge during the revision.

Preliminary contacts have been made with the following representatives of the DOD and USAF for selecting members of the Working Group and discussing revision plans:

T. R. Casberg, Office of the Assistant Secretary of Defense, DOD, Washington, D. C.

E. C. Carter and H. E. Miner, Headquarters, AFLC, USAF, Dayton, Ohio.

Publications

1. R. T. Dillon (1741), Diane Holdridge (5162) and R. Puleo and G. Oxborrow, PHS, Cape Kennedy, Florida, "A Computerized Bacterial Identification System as Applied to Planetary Quarantine," Space Life Sciences, Vol. 3, No. 1, April 1971.
2. M. C. Reynolds, "Investigations of the Synergistic Effects of Thermoradiation on B. subtilis var. niger Spores," presented at the Annual Seminar of the American Society for Microbiology was requested for publication in the Clinical Laboratory Forum, New York, New York.
3. H. D. Sivinski and M. C. Reynolds, "Synergistic Characteristics of Thermoradiation Sterilization," SC-DC-714065, presented at the COSPAR Meeting will be published in COSPAR Proceedings.

Presentations and Briefings

1. H. D. Sivinski spoke to a joint meeting of the local chapter and the visiting Dayton, Ohio chapter of the AIAA on April 13 on "Planetary Quarantine." The meeting was held at the Kirtland Officers Club.
2. R. E. Trujillo (1742) and V. L. Dugan (1733) were judges at the High School State Science Fair in Las Cruces, New Mexico on April 17.
3. A. L. Roark spoke to a class in the Geography Department, University of New Mexico, on April 20. His topic was, "Mathematical Modeling and Analysis of Environmental Systems."
4. H. D. Sivinski, R. T. Dillon, M. C. Reynolds, R. E. Trujillo and V. L. Dugan attended the Annual Meeting of the American Society for Microbiology in Minneapolis, Minnesota on May 2-7. The following presentations were made:
   M. C. Reynolds, "Investigations of the Synergistic Effects of Thermoradiation on B. subtilis var. niger Spores."
   R. E. Trujillo, "Synergistic Effects of Thermoradiation on Microbial Cell Components."
   V. L. Dugan, "A Biomathematical Description of Thermoradiation Inactivation of Bacterial Cells."
5. H. D. Sivinski acted as Moderator of the two day Symposium on Clean Room Technology in Surgery Suites held at the John F. Kennedy Space Center, Florida on May 21 and 22, sponsored by

NASA and Midwest Research Institute. W. J. Whitfield presented a briefing entitled, "Principles of Laminar Airflow Systems," at the same symposium.

6. H. D. Sivinski, W. J. Whitfield and D. M. Garst attended the Tenth Annual Meeting of the American Association for Contamination Control held in Washington, D. C. on May 26. W. J. Whitfield presented a briefing entitled, "The Effect of Relative Humidity on Adhesion of Small Particles to Surfaces."

7. H. D. Sivinski, W. J. Whitfield, R. E. Trujillo and C. A. Trauth, Jr. attended the Semi-Annual NASA Spacecraft Sterilization Technology Seminar in Seattle, Washington on June 18 and 19. The following presentations were given:

   H. D. Sivinski, "Thermoradiation Experimentation."

   W. J. Whitfield, "Fine Particle Physics."

   R. E. Trujillo, "Synergistic Inactivation of Biological Systems by Thermoradiation."

8. H. D. Sivinski attended the PQAP meeting in Seattle, Washington on June 20. Mr. Sivinski presented a briefing entitled, "Thermoradiation Experimentation With Hardy Soil Spores."

9. H. D. Sivinski presented an invited paper, "Synergistic Characteristics of Thermoradiation Sterilization," on June 21 to the Committee on Space Research, International Council of Scientific Unions (COSPAR) in Seattle, Washington.

Additional Activities

1. D. M. Garst, Chairman of the Technical Information Center Committee of the American Association for Contamination Control presided at committee meetings in Washington, D. C. on May 24-25, 1971.
2. D. M. Garst was installed as National Secretary of the American Association for Contamination Control at the Association's Tenth Annual Technical Meeting in Washington, D. C. on May 26, 1971.
3. J. P. Brannen has been invited to become a national lecturer in the Visiting Scientists Lectureship Program for the academic year 1971-72. This program is sponsored by the Society for Industrial and Applied Mathematics (SIAM).

Distribution:

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B. W. Colston, Director
Space & Special Programs Division
Office of Operations
U. S. Atomic Energy Commission
Albuquerque, New Mexico 87115

L. P. Daspit, Jr.
Viking Project Quarantine Officer
Viking Project Office, NASA
Langley Research Center
Hampton, Virginia 23365

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Richard G. Bond
School of Public Health
College of Medical Science
University of Minnesota
Minneapolis, Minnesota 55455

John H. Brewer
Mountain View Road
Star Route 2
Brownwood, Texas 76801

Edward B. Kasner
Director of Research Services
Graduate College
University of New Mexico
Albuquerque, New Mexico 87106

Frank B. Engley, Jr., Chairman
Department of Microbiology
School of Medicine
University of Missouri
Columbia, Missouri 65201

Gilbert V. Levin
Biospherics, Inc.
4928 Wyaconda Road
Rockville, Maryland 20853

Irving J. Pflug
Professor of Environmental Health
545 Space Science Center
University of Minnesota
Minneapolis, Minnesota 55455

Gerald J. Silverman
Department of the Army
U. S. Army Natick Laboratories
Natick, Massachusetts 01760

John A. Ulrich
Department of Microbiology
School of Medicine
University of New Mexico
Albuquerque, New Mexico 87106

Samuel Schalkowsky
Exotech Systems, Inc.
525 School Street S. W.
Washington, D. C. 20024

Mark A. Chatigny
Research Engineer
Naval Biological Laboratory
Naval Supply Center
University of California, Berkeley
Oakland, California 94625

Richard G. Cornell
Associate Professor of Statistics
Department of Statistics
Florida State University
Tallahassee, Florida 32306

Dr. Richard C. Corlett
Department of Mechanical Engineering
University of Washington
Seattle, Washington 98105

Martin S. Favero
Department of Health, Education,
and Welfare
CDC-Phoenix Field Station
4402 North 7th Street
Phoenix, Arizona 85014

Mr. James Martin
Viking Project Engineer
Langley Research Center, NASA
Langley Station
Hampton, Virginia 23365

Q. Ussery
Code NC3, Quality Assurance Branch
Manned Spacecraft Center, NASA
Houston, Texas

F. J. Beyerle
George C. Marshall Space Flight
Center
Manufacturing Engineering Laboratory
Code R-ME-MMC
Huntsville, Alabama 35812

J. Gayle
Code SOP
Kennedy Space Center, NASA
Cape Canaveral, Florida

Murray Schulman
Division of Biology and Medicine
Headquarters, AEC
Washington, D. C. 20545

N. H. MacLeod
Space Biology Branch
Code 624, Bldg. 21, Room 161
Goddard Space Flight Center
Greenbelt, Maryland 20771

Jeptha E. Campbell, Ph.D., Chief
Division of Microbiology
Food and Drug Administration
DHEW, Public Health Service
1090 Tusculum Avenue
Cincinnati, Ohio 54226

G. Rotariu
Process Radiation Staff
Division of Isotopes Development
Headquarters, AEC
Washington, D. C. 20545

Martin G. Koesterer, Microbiologist
Bioscience Operation
Valley Forge Space Technology Center
P. O. Box 8555
Philadelphia, Pennsylvania 19101

Carl W. Bruch, Chief,
Drug Microbiology Branch
BD415
Food and Drug Administration
200 C Street S. W.
Washington, D. C. 20204

John W. Beakley
Department of Biology
University of New Mexico
Albuquerque, New Mexico 87106

Loren D. Potter, Chairman
Department of Biology
University of New Mexico
Albuquerque, New Mexico 87106

Loris W. Hughes
Department of Biology
New Mexico State University
University Park, New Mexico

Richard W. Porter
Corporate Engineering Staff
General Electric Company
570 Lexington Avenue
New York, New York 10022

Fred L. Whipple
Smithsonian Astrophysical Observatory
Cambridge, Massachusetts 02138

J. J. McDade
Environmental Research Laboratory
Building 1710
Dow Chemical Company
Midland, Michigan 48640

Otto Hamberg
Aerospace Corporation
Building A2, Room 2019
2350 East El Segundo Blvd.
El Segundo, California

Lawrence P. Chambers
NASA Headquarters
Office of Manned Space Flight
Code MLR
Washington, D. C. 20546

Arthur H. Neill
Code SL
400 Maryland Avenue S. W.
Washington, D. C. 20546

Richard H. Green
Sterilization Group
Jet Propulsion Laboratory
4800 Oak Grove Drive
Pasadena, California 91103

Rudy Puleo
Spacecraft Bioassay Unit
Center for Disease Control
USPHS
Cape Kennedy, AFS, Florida 32900

USAEC, Division of Technical
Information
P. O. Box 62
Oak Ridge, Tennessee 37830
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Los Alamos Scientific Laboratory
Los Alamos. New Mexico 87544

Jack Kaye
11607 Georgetowne Court
Potomac, Maryland 20854

Dr. Robert Angelotti
Office of Food Sanitation, FDA
200 C Street S. W. (BF-201)
Washington, D. C. 20204

Vance I. Oyama, Chief
Life Detection Systems Branch
NASA, Ames Research Center
Moffett Field, California 94035

Byron W. Brown, Jr.
Department of Preventive Medicine
Stanford University School of Medicine
Stanford University Medical Center
Palo Alto, California 94304

Don G. Fox
Sterility Control Officer
NASA Headquarters, Code SL
400 Maryland Avenue S. W.
Washington, D. C. 20546

A. A. Rothstein
Manager, Planetary Quarantine
Viking Program
Martin Marietta Corporation
P. O. Box 179
Denver, Colorado 80201

Hillel S. Levinson
U. S. Army Natick Laboratory
Natick, Massachusetts 01760

Dr. Walter M. Urbain
College of Agriculture
Michigan State University
East Lansing, Michigan 48823

H. O. Halvorson
Biochemistry Department
St. Paul Campus
University of Minnesota
St. Paul, Minnesota 55414

A. Anellis
U. S. Army Natick Laboratories
Natick, Massachusetts 01760

H. W. Johnson, LTC
U. S. Army Medical Research and
Development Command
Washington, D. C. 20314

Donald A. Kautter
Department of HEW
Food and Drug Administration
Division of Microbiology
BF-135
200 C Street S. W.
Washington, D. C. 20204

Lt. Keith C. Hopkins (SAH)
Biomedical Group
Air Force Weapons Laboratory
Kirtland Air Force Base
Albuquerque, New Mexico 87115

Briggs Phillips
Becton, Dickinson Research Center
P. O. Box 11276
Raleigh, North Carolina 27604

Dr. Wolf Vishniac
Department of Biology
University of Rochester
Rochester, New York 14627

F. A. Leone, Program Manager
Radiation Preservation of Foods
Division of Isotope Development
AEC Headquarters, Mail Sta. 255
Washington, D. C. 20545

Dr. Orr Reynolds
American Physiological Society
2134 LeRoy Place N. W.
Washington, D. C. 20008

J. A. Hornbeck - 1
J. M. Wiesen - 100
W. J. Howard - 1000
D. B. Shuster - 1200
C. B. McCampbell - 1310
W. A. Gardner - 1500
H. E. Lenander - 1600
T. M. Burford - 1700
C. Winter - 1710
T. M. Burford (Actg.) - 1720
J. W. Worrell, Jr. - 1721
D. P. Peterson - 1724
R. G. Clem - 1730
H. D. Sivinski - 1740 (35)
A. A. Lieber - 1750
B. H. Van Domelen - 1913
A. M. Clogston - 5000
L. C. Hebel - 5200
J. V. Walker - 5220
R. M. Jefferson - 5221
J. E. McDonald - 5300
L. M. Berry - 5500
R. E. Henderson - 7000
L. S. Ostrander - 8232
G. A. Fowler - 9000
J. H. Scott - 9200
L. Hollingsworth - 9300
L. A. Hopkins, Jr. - 9400
D. W. Ballard - 9461
J. L. Gardner - 3142
R. S. Gillespie - 3151

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